Prevalence and Symptom Correlation of Abnormal Breath Hydrogen Tests in Patients with Irritable Bowel Syndrome

A dissertation submitted in partial fulfillment of the requirements for
DM (Medical Gastroenterology) examination of the
Tamil Nadu Dr. M.G.R. Medical University, Chennai,
to be held in August 2014.

Certificate

This is to certify that this dissertation entitled **"Prevalence and Symptom Correlation of Abnormal Breath Hydrogen Tests in Patients with Irritable Bowel Syndrome"** is a bonafide work done by Dr. Laxmikant Desai, Christian Medical College (CMC), Vellore in partial fulfillment of the rules and regulations for DM (Medical Gastroenterology) examination of The Tamil Nadu Dr MGR Medical University, to be held in August 2014.

Dr. B.S.Ramakrishna
(Guide)
Professor
Dept of Gastroenterology
CMC, Vellore
Date:

Dr.C.E.Eapen
Prof and Head
Dept of Clinical Gastroenterology and Hepatology,
CMC Vellore
Date:

Principal/Dean
Christian Medical college
Vellore
Date:

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Dear Mr. Denzil,

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Prevalance and symptom correlation of abnormal breath hydrogen test in patients with irritable bowel syndrome.
Dr. Laxmikant Desai, Senior Registrar, Medical Gastroenterology,
Dr. B.S. Ramakrishna, Dr. Ebby George Simon, Dr. Sudipta Dhar Choudhary, Medical Gastroenterology

Ref: IRB Min. No. 7930 dated 02.08.2012

The Institutional Review Board at its meeting held on 2nd August, 2012 vide IRB Min. No. ***7930*** accepted the project for *2Years* at a total sanction of *Rs.80, 000/- (Rupees Eighty Thousand Only) A sum of Rs 40,000/- will be sanctioned for 12 months after receipt of the revised proposal, subsequent installment of 40,000/- each will be released at the end of the first year following the receipt of the progress report*. Kindly arrange to transfer the sanctioned amount to a separate account to beoperated by Drs. Laxmikant Desai & B.S. Ramakrishna

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Institutional Review Board

CC: Dr. Laxmikant Desai, Senior Registrar, Medical Gastroenterology, CMC
Dr. B.S. Ramakrishna, Medical Gastroenterology, CMC
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CONTENTS

Introduction

Irritable bowel syndrome (IBS) is defined as "a functional bowel disorder in which abdominal pain or discomfort is associated with defecation or a change in bowel habit, and with features of disordered defecation"[1]. IBS is a relapsing functional bowel disorder which is diagnosed based on criteria including symptoms in those without any detectable organic causes. It is the most common gastrointestinal disorder diagnosed and accounts for around 30 percent of all referrals to gastroenterologists[2]. Previously diagnosis of IBS was made after exclusion of all organic causes. After the introduction of new and more precise clinical criteria, i.e. "Rome criteria", it has allowed to make a diagnosis of IBS on the basis of symptoms alone without extensive evaluation[3].

In 1978 *Manning et al* formulated a group of symptoms to diagnose IBS. These were called as Manning criteria for diagnosis of IBS[4]. Soon various definitions for IBS were introduced. Hence to standardize the definition of IBS especially for research, an international working group published a consensus definition in 1992 which is called as the Rome criteria, last revision of which was done in 2005[1]. Of these most commonly used criteria in clinical practice and various studies are Rome II and III.

IBS is considered to be the most common of all gastrointestinal disorders which has variable prevalence of 5-25 % worldwide. In a prospective, multi-centre study by *Ghoshal UC et al*, data was collected from 2785 patients who presented with chronic lower gastrointestinal symptoms and concluded that symptoms suggestive of IBS was present in 4.2% in community subjects [5].In another study which included 2,549 subjects from an urban community from Western part of India, *Shah et al* reported the prevalence of IBS as 7.5% using Manning criteria[6]. The prevalence of IBS from population-based studies in North

America estimated is around 10 to 15 percent[6,7]. A community based study from Europe showed a prevalence of 11.5 %, however, the prevalence was variable among different countries[8].

IBS can affect both males and females including elderly. However, IBS is more common in younger population. In a systematic review from North America, there was female predominance with overall ratio of female: male 2:1 [9]. Studies from India have been varying, but majority have shown male predominance [5,6]. Patients with IBS present with various symptoms which may be gastrointestinal or extra intestinal complaints. However, the symptom of prolonged pain abdomen with altered bowel habits is the primary characteristic of IBS but is non specific. Abdominal pain is usually mild and has waxing and waning character. There is wide variation in location and intensity of pain in patients with IBS [1,10]. Patients present with altered bowel habits, which range from diarrhoea, constipation, alternating diarrhoea and constipation, or normal bowel habits alternating with either diarrhoea and/or constipation. Depending on predominant symptoms IBS is sub classified into diarrhoea predominant (IBS-D), constipation predominant (IBS-C) and Mixed or Intermediate (IBS –I).

Despite high prevalence in general population the path physiology of IBS remains uncertain. A number of mechanisms have been implicated in the pathogenesis of IBS, which include altered GI motility, visceral hypersensitivity, altered gut flora, low-grade inflammation, stress among the others[11,12]. Small bowel and colonic transit time has been documented to be delayed in IBS-C and accelerated in IBS-D[8]. It is shown that IBS develops in about 7% to 30% of those subjects who have recovered from an episode of bacterial enteritis [13]. It has been postulated that the colonic flora could be abnormal in some of IBS subjects, which

results in increased colonic fermentation, excess production of gas, and development of symptoms associated with the disease[14]. One of the associations of IBS which has been proposed is with small intestinal bacterial overgrowth (SIBO).

SIBO is a condition which is characterised by increased number of bacteria (>10^5 bacteria/ml in jejunal fluid), in small intestine which predominantly include species commonly present in colon. Whether or not SIBO contributes to some of the symptoms in IBS such as gas and bloating is an area of active investigation. The gold standard used for the diagnosis of SIBO includes demonstration of more than 10^5 colony forming units(CFU)bacteria/ml of fluid from jejunal aspirate. Non-invasive breath tests are commonly used to diagnose SIBO in clinical practice. These tests are based on detection of gases like hydrogen or methane, which are produced by bacteria in intestines after metabolism of carbohydrates like glucose or lactulose.

In a study by *Pimentel et al.* abnormal breath test results suggesting SIBO was found in 78%[15]. In another study by the same group the incidence of an abnormal lactulose breath test in subjects with IBS (as per Rome II) was 84% vs. 20% in the control of subjects [16]. The normalization of the lactulose BT after the use of antibiotics along with a significant reduction of IBS symptoms was also noted in the same study.

A study by *Rana SV* et al. showed that the prevalence of SIBO in IBS patients from North India was 11.1% [17]. In a study by *Ghoshal UCet al* the noted prevalence of SIBO was 8.5% [18] and in a study by *Sachdeva S et al* it was 23.7%[19].

In addition to constipation, diarrhoea, or pain many of the IBS patients also present with a significant bloating component to their presentation. Recent data suggest that abnormalities in

gas production and its transit through the small intestine could explain these symptoms[20]. Whether SIBO contributes to some of the distressing symptoms such as gas and bloating in IBS remains to be established.

The association between SIBO and IBS continues to be controversial with prevalence in different parts of the world being apparently quite different. There is a paucity of studies from south India. Hence the present study was planned to find out the prevalence of SIBO in IBS patients using glucose hydrogen breath test (GHBT). The study also aimed to evaluate symptom correlation of SIBO in these patients with abnormal GHBT. The patients were also planned to undergo lactulose hydrogen breath test (LHBT) to estimate orocecal transit time (OCTT) and to analyse if it was different in different subgroups of IBS.

AIMS

The primary objective of the present study was to study prevalence of abnormal glucose hydrogen breath test, as a marker for Small Intestinal Bacterial Overgrowth (SIBO), in patients with Irritable Bowel Syndrome (IBS) and to correlate with the symptoms.

The study also aimed to find out the orocecal transit time in these patients using the lactulose hydrogen breath test.

Review of Literature

Definition

Irritable bowel syndrome (IBS) is defined as "a functional bowel disease in which abdominal discomfort or pain that is associated with a change in bowel habit. "[1] .

A diagnosis of IBS is based on symptoms which are consistent with the condition, and after exclusion of other conditions which present with similar clinical presentation in a cost-effective manner. These conditions include any other organic or functional disorders[8]. This strategy was recommended by 2009 American Gastroenterology Association (AGA) practice guidelines.

Diagnostic Criteria for IBS

There have been multiple diagnostic criteria which have been described previously.

In 1978 *Manning et al* formulated a symptom complex suggestive of IBS, which are referred to as Manning criteria[4]. By using these criteria, studies which were done showed that this was more specific in identifying the patients with IBS but was less sensitive. These criteria were of greater diagnostic value in women as compared to men [21].

Manning criteria for IBS are as follows

- Abdominal pain eased after bowel movement
- Looser stools at onset of abdominal pain
- More frequent bowel movements at onset of abdominal pain

- Abdominal distension
- Mucus per rectum
- Sensation of incomplete emptying

The Kruis scoring system[22] was the other diagnostic criteria which were used for the diagnosis of IBS. The criteria were based on the history of the patients, assessment by the physician including physical examination and simple blood tests. As per the criteria a diagnosis of IBS was not considered if there were abnormal findings on physical examination, low haemoglobin (<14 g/dl in males: <12 g/dl in females), high total leucocyte count (>10,000/mm^3) or raised ESR (>20 mm at 2 hours). It had modest diagnostic utility.

As there were multiple criteria were being used for diagnosis of IBS, Rome criteria were established in order to standardise the diagnosis of IBS especially for clinical research purposes in 1992. Till date there are Rome I, II and III criteria are described with latest revision being in 2005. The sensitivity and specificity of the Rome I criteria have been reported to be 71% and 85%, respectively[23]. The main components of Rome II and III criteria are very similar but adequate validation of Rome III has not been done.

The Rome II diagnostic criteria[24] for IBS:

These are already described in detail under the heading of methods in the earlier section. Depending on the predominant symptom IBS can be subdivided into three groups which include IBS –D, IBS-C and IBS-I.

The Rome III diagnostic criteria[1] for IBS:

Recurrent abdominal pain or discomfort at least three days/month in the last three months associated with *two or more* of the following:

- Improvement with defecation
- Onset associated with a change in frequency of stool
- Onset associated with a change in form (appearance) of stool

Based on Bristol stool form, IBS patients are sub classified in to 4 types which is presented in table no 1 [1].

Table no. 1: IBS subtypes as per Rome III

1	IBS - C	hard stools ≥ 25%* and watery stools < 25 %
2	IBS - D	watery stools ≥ 25 % and hard stools < 5%
3	IBS - M	watery stools ≥ 25 % and hard stools ≥ 25%
4	IBS - U	who do not fit into other subtypes

C- constipation, D – diarrhoea, M – Mixed, U – unsubtyped,
* % based on number of bowel movements

Epidemiology of IBS

IBS is considered as the most common functional bowel disease worldwide. There is a considerable variation in epidemiology of IBS especially in studies from India and west.

In a study by *Shah et al* which was published in 2001 reported the prevalence of IBS as 7.6%. The study was a community based one which used Manning criteria for diagnosis of IBS. In this study total of 2549 apparently healthy adults (mean age 37.2 years; 1441 men) were interviewed using a detailed symptom questionnaire[6].

A prospective multicentre study was conducted by Indian society of Gastroenterology task force and was published in 2008. In this study data were collected from 2785 subjects who presented with chronic lower GI symptoms in 30 centres and 4500 asymptomatic community subjects, using separate questionnaires. The study reported that symptoms complex suggestive of IBS was present in 4.2% in community population. The study also showed slight male predominance in prevalence of IBS with 4.3% in males as compared to 4% in females[5].

A prospective study was done by *Makharia et al* which was published in 2011. The study included 4767 subjects from rural north India who were interviewed with questionnaire of IBS. Using Rome III criteria, 191 patients were diagnosed with IBS. The prevalence of IBS in the study was 4%. In the study IBS-M was the most common presentation with 42.4%. IBS-D, IBS-U and IBS-C were diagnosed in 37.7%, 13.6% and 6.3% respectively. In this study, mean age was 34.6 (± 10.8) years. Females had a higher prevalence of IBS with 4.8% as compared to 3.2% in males [25].

The prevalence of IBS from population-based studies in North America estimated is approximately 10 to 15 percent. Male to female ratio was 1:2 [7,8]. A community-based study from Europe showed a prevalence of 11.5 %, however, it varied widely among countries[9].

As can be seen from the data presented above, it can be noted that the prevalence of IBS in India is much lower than that reported from the West. The prevalence of IBS in India is also much lesser as compared to other population based studies from other countries from Asia like China (11.5% by Manning criteria)[26], Bangladesh (8.5% by Rome II criteria)[27], Pakistan (14% by Rome II criteria)[28].

The incidence of irritable bowel syndrome is uncertain. A study from Olmsted County in United States of America by *Locke III GR et al* reported that the incidence of IBS in was 0.2% per year based on clinical diagnosis[29].

Pathogenesis of IBS

Despite high prevalence exact aetiology and pathogenesis of IBS remains unknown. Multiple mechanisms have been described in the etiopathogenesis of IBS, which include altered intestinal motility, low-grade inflammation, genetic factors, dietary factors, abnormal expulsion of gas, visceral hypersensitivity, and stress among the various others. Genetic factors are postulated to alter the processing of the inflammatory and immune responses locally and GI signals centrally which can predispose to IBS. Hence it may be reasonable to postulate that “multiple hits” rather than a single mechanism is required to manifest IBS [2,11]. Various factors are explained in brief in the following section.

Altered colonic and small bowel motility

Symptom of diarrhoea in functional bowel diseases like IBS can be explained by multiple mechanisms like increased high-amplitude propagated contractions (HAPCs), increased motor activity in recto sigmoid region soon after meal which is called gastro colic response,

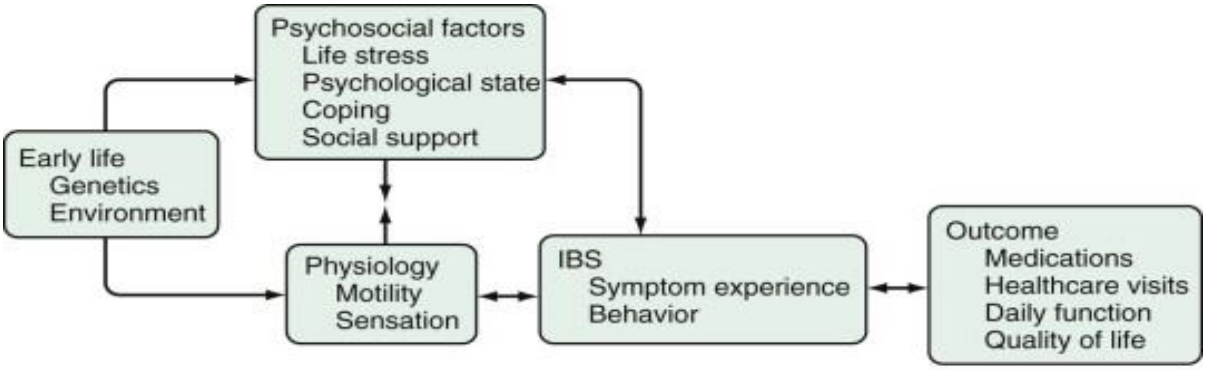


Fig no.2: A conceptual model of IBS which depicts relationship between various factors [2]

or rectal hypersensitivity. Constipation in these subjects could be explained by increased non propulsive contractions, reduced number of HAPCs, or impaired rectal sensation[30]. Previous studies have shown that small intestine and colonic transit is delayed in IBS-C and accelerated in IBS-D [31]. Increased HAPCs can also explain abdominal pain. It has also been demonstrated in prior studies that colonic transit is accelerated by stress or instillation of bile acid like deoxycholic acid, but such alteration in transit is not specific for IBS subjects[8].

Visceral hypersensitivity

In a study by *Ritchie J et al*, balloon in rectum was inflated at various volumes in IBS patients and controls. It was shown in the study that balloon distension induced pain at lower volumes in patients with IBS which suggests that hypersensitivity of rectum or colon may be involved in pathogenesis[32]. It is not a universal phenomenon but was seen in 60% of patients

of IBS, hence can't explain the symptom in all patients. It has been demonstrated that patients of functional bowel disease are more likely to appreciate intestinal contractions or gas as compared to others after meal or in association with stress. Various neurotransmitters are known to be involved in visceral hypersensitivity, among which serotonin is most important[33].

Abnormal dynamics of gas

It is demonstrated in studies that abdomen normally swells during the day, which attains its peak in the evening and decreases on lying down during abdominal girth monitoring of ambulatory subjects. One of the mechanisms which has been proposed in pathogenesis of IBS is that, such a phenomenon may often be exaggerated in subset IBS subjects, which may explain bloating [34]. Following infusion of gas in small bowel, it was noted that IBS subjects retained more gas than controls in a study. When asked not to pass gas voluntarily after infusion of gas, IBS subjects felt more discomfort as compared to healthy controls [35].

Local inflammation

The human gastrointestinal tract (GIT) is in a state of chronic inflammation normally, as a result of constant interaction between normal enteric bacteria and the mucosal immune system. It has been demonstrated that inflammatory cells, including mast cells and T lymphocytes are present in higher numbers in the mucosa of a subgroup of IBS patients, which may suggest that an ongoing low-grade inflammation may be present in these patients. Nearly 7% to 30% of patients after recovering from a documented episode of enteritis caused by bacteria, have been demonstrated to develop IBS subsequently. This is described as post-

infectious IBS. The risk of this increases, if the episode of bacterial enteritis is longer than three weeks or it is caused by the organisms which are toxigenic[36] There is evidence of increased production of serotonin, arachidonic acid metabolites and adenosine in areas of colonic inflammation in IBS. There is also increase in various chronic inflammatory and enteroendocrine cells. It has also been demonstrated that increased intestinal permeability by using the lactulose-mannitol test in patients with post-infectious IBS[37].

Role of food

Many patients with IBS complain about worsening of symptoms after consumption of certain foods which include wheat, egg, dairy products, and onions among others. One of the common associations with IBS is intolerance after consumption foods containing wheat. In a study involving IBS-D patients who were positive for HLA DQ2 symptoms in 70% of patients as compare to 20% who were negative , after a 6 month gluten-free diet [38].

FODMAP is an acronym for fermentable oligosaccharides, disaccharides, monosaccharides and polyols. These are dietary constituents which are poorly absorbed, and produce short-chain fatty acids in the small bowel. These short chain fatty acids may induce symptoms of IBS through their effects on colonic motility and secretion[39]. It is also shown in various studies that the symptoms of IBS improve after avoidance of these ingradients in the diet. Patients of lactose intolerance may also have symptoms which overlap with those with IBS. In a study by *Gupta D et al* from north India demonstrated that the frequency of lactose intolerance was high among IBS patients (n=89, as per IBS II) and comparable with control subjects (n=53), was 72% and 60% respectively which was statistically insignificant. The study also concluded that there was poor sensitivity of self-reported intolerance to milk in

detecting lactose intolerance[40]. Malabsorption of fructose or sorbitol has been shown to contribute to few symptoms of a subset of IBS subjects; but this mechanism is unlikely to be more common given a high prevalence of IBS[41].

Altered central regulation

Normally afferent signals from gastrointestinal tract reach thalamus via brainstem but such signals are consciously perceived only rarely. Alteration and modulation of these afferent signals can occur at various levels which include enteric, spinal, and central levels. Studies which are based on functional brain imaging that detect changes in blood flow, have suggested that after visceral stimuli, responses in brain are varied in subjects of IBS as compared to healthy controls. In a study with IBS subjects, after delivered or anticipated rectal distension, there was higher activation of the mid-cingular cortex. These observations suggest why stress or anxiety can increase the perception of visceral pain and relaxation can decrease perception of pain in subjects with IBS[42].

Psychological factors

As many as 40 to 94% of patients with IBS have associated anxiety, depression and somatisation, which are the most common psychiatric conditions associated with IBS [43]. A childhood history of abuse is often noted in patients with IBS than in those without which may be emotional or physical including sexual. Altered central brain modulation in response to pain can explain in some of these patients. Subjects with IBS more commonly report a higher lifetime and daily events with stress than healthy people or those having organic diseases [8].

Genetic factors

Hereditary component in IBS is increasingly being recognised but evidence is limited. Twin studies have shown higher concordance in monozygotic as compared dizygotic twins in patients with IBS. Various candidate genes have been postulated to be associated with IBS, but further studies and investigations are required before their functional significance is understood[44].

There has been varied association between SIBO and IBS which is reviewed in the following section.

Small intestinal bacterial overgrowth (SIBO)

SIBO is defined as a disorder where there are increased numbers of bacteria, which are mainly colonic type ($>10^5$ bacteria/ml in jejunal fluid), present in the lumen of the small intestine[45]. In SIBO multiple organisms are present in varying numbers. Common species include *Streptococci, Bacteroides, Escherichia, and Lactobacillus*[46]. The upper small bowel contains relatively low bacterial counts because of increased acid content and normal peristalsis as compared to distal intestine. Normally upper small bowel shows bacterial count lower than 1000 per ml of aspirate.

The aetiology and proposed pathogenesis is presented in table no 2. IBS is one of the proposed causes of SIBO but the exact pathophysiology is not known.

Table no. 3: Pathophysiology and conditions associated with SIBO

Pathophysiology	Causes
Abnormal anatomy	Blind loop syndrome – post surgical Jejunal diverticulosis Stricture of small bowel Gastrocolic or Enterocolic fistula
Altered motility	Diabetes mellitus Connective tissue diseases like Scleroderma Idiopathic
Reduced gatric acid	Post vagotomy state Drugs – Ex: proton pump inhibitors Atrophic gastritis
Multiple mechanisms – known or unknown	IBS Chronic pancreatitis Chronic liver disease Chronic kidney disease

The gold standard method used for the diagnosis of SIBO involves demonstration of $>10^5$ colony forming units (CFU) of bacteria/ml of jejunal fluid. Unfortunately, such aspiration is invasive and time-consuming. It has been argued that this method may not detect bacterial overgrowth if it involves only distal small intestine. In a study by *Corazza* and colleagues, when intestinal fluid was assessed by collection at two different sites in jejunum, bacterial counts showed a good correlation [47]. Problems commonly encountered during jejunal aspirate are (a) contamination with oral organisms during aspiration (b) technical difficulty and, (c) availability of media required for transport and culture of aspirate.

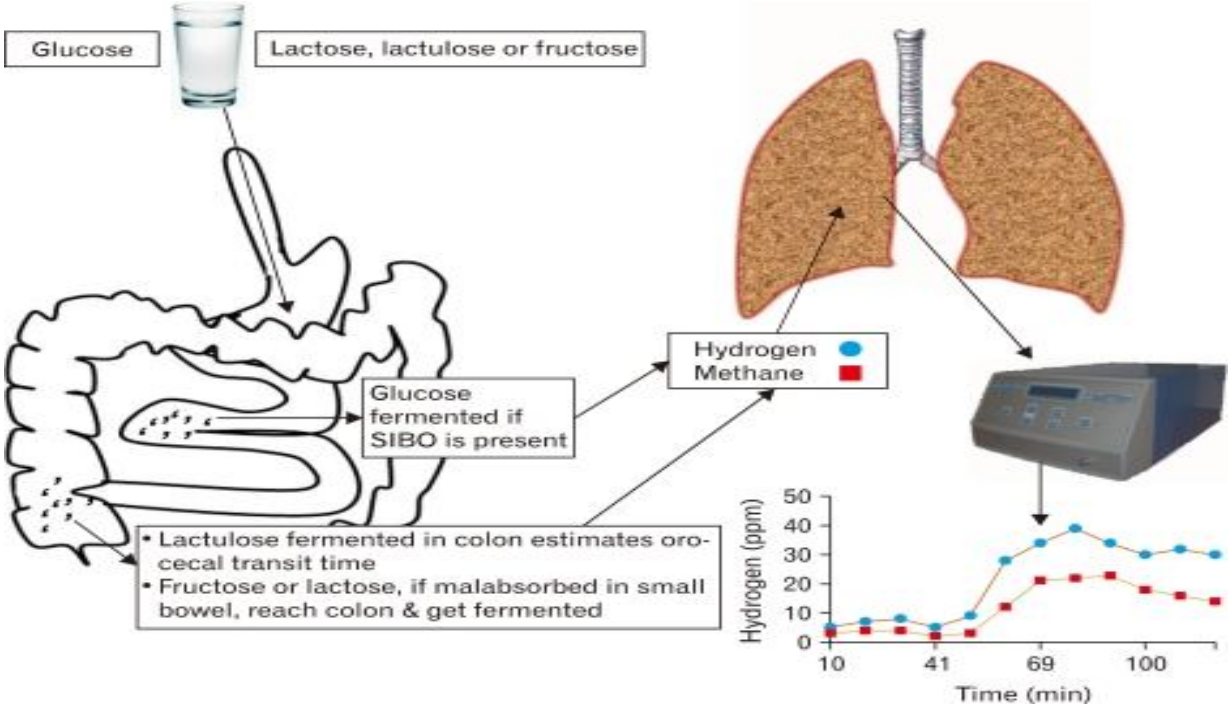
In order to overcome these problems, various non invasive tests have been developed for diagnosing SIBO which include the breath tests. Hydrogen or methane gas is produced when glucose or lactulose is metabolised by the bacteria in intestine. Majority of it is consumed by bacteria and only a small fraction of this gas is excreted by the various routes. The hydrogen which is excreted via lungs is detected in the breath which forms the basis of these tests. These HBTs are based on basic principle that other than the metabolism of undigested carbohydrates there is no other source of hydrogen gas in human body [48]. The principle of breath test is shown in figure no. 2. Hydrogen or methane detected in breath are expressed in parts per million (ppm).

There are several potential problems which are associated with conduct of breath tests. Approximately 15% of the healthy subjects are considered as predominant methane producers. These indivisual's intestines are colonized with *Methanobrevibacter smithii*. In these persons hydrogen gas produced reacts biochemically with carbon dioxide to produce methane. This leads to less hydrogen being produced in these persons as compared to others and hence hydrogen may not be detected in breath tests and predominantly methane is detected. Altered intestinal motility may affect the accuracy of these tests. The result of breath tests, especially the baseline value, will also be affected by recent consumption of diet which has high content of non digestible carbohydrates by the persons undergoing these tests, smoking and physical exercise prior to test [49].

For detection of SIBO, glucose HBT was shown to have sensitivity varying from 27% to 52% and specificity of 30% to 83% in the previous studies. It has been shown that with altered

intestinal motility results of LHBT are difficult to interpret. This leads to varying sensitivity and specificity for LHBT [47].

Fig no. 3: Figure which depicts principle of HBTs[50]



IBS and SIBO

Many patients with SIBO fulfil the diagnostic criteria for IBS because symptoms of both of these conditions overlap. Bacterial flora in small intestine of a subset of IBS subjects could be abnormal which results in increased fermentation leading to excessive gas [14]. It has been postulated that this leads to symptom of bloating. Based on these observations, there have been multiple studies on their association and the clinical trials of improvement in symptoms with use of antibiotics or probiotics.

In a study of 202 patients, who met Rome I criteria for IBS, by *Pimentel et al* using lactulose HBT, SIBO was found in 157 (78%) of patients. Out of these 202 patients 76.7% were females and mean age was 41.9 (± 15.2) years. LHBT was done using 10 g of lactulose in the study and was taken as positive if hydrogen level was more than 20 ppm. In those who had abnormal HBT, 10 day course of antibiotics were administered as per discretion of physician. The study also showed that use of antibiotic therapy reduced hydrogen production and improved symptoms [15].

In another study by the same group LHBT was 84% (93/111) in IBS patients as against 20% (3/15) in healthy people. IBS was diagnosed using Rome I criteria in the study. This study was a randomised control trial with 111 IBS subjects of which 55 were treated with neomycin, 56 with placebo. The study showed that there was significant improvement in compostite score which was used in study and normalization of bowel habits in those treated with neomycin than those with placebo [16].

In a study from Italy, a total of 65 IBS subjects (as per Rome II) and 102 healthy subjects were enrolled. Glucose HBT was done with 50 g and positive HBT taken as >10 ppm above baseline. Positivity GHBT was noted in 31% of IBS and 4% in the control population. This was statistically significant [51].

A study by *Rana SV* et al. from PGI, Chandigarh showed that the prevalence of SIBO was 11.1% in IBS subjects. The study included 225 patients with IBS (as per Rome II criteria) and 100 healthy people as control population. Out of these patients with IBS, 71.1% were

males. Mean age for male patients was 43.5 (20-65) yrs and 48.7 (16-60) yrs for females. GHBT was done with 50 g and positive HBT was taken when hydrogen in breath exceeded 12 ppm above baseline. This study concluded that SIBO was more common in IBS patients (11.1%) as compared to controls (1%), which is lower than the reported prevalence elsewhere[17].

In a study by *Ghoshal UC* prevalence of SIBO in IBS was 8.5%. In this study 129 patients of IBS as per Manning's criteria, 73 with chronic non specific diarrhoea, CNSD (defined as diarrhoea for ≥ 4 weeks, with atleast 2out of 3 normal test results of urine xylose, syool fat and D2 biopsy) and 51 control subjects were included. They were evaluated for SIBO using glucose HBT. Majority of IBS patients were males (83.7%) and age was 36.6 (± 11.4) yrs. GHBT was done with 100 g and positive HBT was diahnosed when hydrogen in breath exceeded 12 ppm above baseline. Of total of 129 patients with IBS, IBS – I was diagnosed in 122 and IBS –C in 7, 20 patients with IBS-D were included in CNSD group. In the study none of patients with IBS-C had SIBO. There was no significant difference in prevalence of SIBO in patients with IBS-D as compared to other subjects with CNSD. This study demonstrated that SIBO using GHBT was commoner in CNSD (21.9%) than in IBS (8.5%) or healthy control subjects (2%) [18].

In a study by *Sachdeva et al* 59 of IBS patients (as per Rome III) and 37 controls were included. Among patients with IBS, 27 were IBS-D subtype, 11 were IBS-C & 21 were M-IBS- M. Median age was 34 (18-74) yrs in these IBS subjects and majority were males (69.5%). GHBT was done with 100 g and abnormal HBT was considered when hydrogen in breath exceeded 12 ppm above baseline. SIBO (using GHBT) was statistically more

common in IBS subjects (23.7%) as compared to healthy controls (2.7%). The study also concluded that IBS-D subtype, female sex & symptom of bloating as probable predictors of SIBO in those diagnosed with IBS[19].

A Swedish study by *Posserud et al* included 161 IBS patients (who fulfilled Rome II criteria) and 42 healthy controls. The mean age of IBS subjects was 38 (19– 68)yrs majority were females (120). Among them 49 patients belonged to IBS- D category, 37 to IBS-C and 76 to IBS-I. A subset of patients also underwent glucose and lactulose HBT. Using jejunal cultures to diagnose SIBO ($>10^5$ /ml), the study showed that frequency of SIBO was same in both IBS patients and healthy controls at 4%.The authors refuted any strong association of SIBO and IBS using gold standard of jejunal aspirate and HBTs [52].

In a meta-analysis by *Ford AC,* published in 2009, included 12 studies with 1921 r IBS subjects. In this study pooled prevalence of a positive LHBT was 54% (95% CI, 32%–76%) and GHBT was 31% (95% CI, 14%–50%). This meta-analysis showed the pooled odds ratio SIBO in comparison to control population was 3.45 (95% CI, 0.9–12.7) or 4.7 (95% CI, 1.7–12.95), based on the criteria used for the study. The authors concluded that the role of testing for SIBO in individuals with IBS remains unclear[53].

In a meta-analysis by *Shah et al* published in 2010 included 11 studies (1,076 patients with IBS and 509 healthy controls). Abnormal HBTs were more common in IBS patients as compared to healthy controls (OR = 4.46, 95% CI = 1.69– 11.80). This association became more apparent when better designed age- and sex-matched studies were taken (OR = 9.64)..

But the studies were heterogeneity in criteria used for selection, substrate used for HBT and cut off used for positive HBT [54].

A number of studies were done to assess the role of antibiotics in treatment of SIBO in patients of IBS. *Pimentel M et al* studied 87 patients with IBS (using Rome I), and were administered 400 mg of rifaximin or placebo thrice a day for a period of 10 days. At the end of 10 week follow up period, comparison of the global improvement scores of rifaximin vs placebo groups demonstrated an average increase of 36.4% vs 21%, respectively[55].

Two studies were designed by TARGET group which were phase 3 double blind RCTs which were called TARGET 1 and 2. In this study IBS subjects without constipation were divided into two groups randomly, one received rifaximin (at 550 mg thrice daily) or placebo for two weeks. After combining both the studies, rifaximin arm had significant relief of global symptoms at 4 weeks as compared to placebo arm (40.7% vs. 31.7%, $P<0.001$). Both the studies individually also had statistically significant difference between the two groups[56].

A study by *Meyrat P et al* included 106 of 150 IBS patients diagnosed with SIBO using LHBT. They were treated with rifaximin (800mg/day) for a total duration of two weeks. All the patients were assessed using a questionnaire of symptom severity on a Likert scale at the onset, 4 and 10 wks after the treatment. This study showed a significant improvement in IBS symptoms at the end of three months. 86% of those undergoing repeat LHBT at 4 weeks had a negative test [57].

Factors that predict SIBO in IBS

Few studies in the past have evaluated the factors that predict SIBO in IBS patients. In a study by *Reddymasu et al*, which was a retrospective study, included IBS patients (as per Rome II criteria) and underwent HBT using 50 g of glucose. Bloating and flatulence were predominant symptoms in all 98 patients of IBS. Among them 52% had IBS-C, 39% IBS-D, and 9% had IBS-I. SIBO was diagnosed in 35 (36%) patients as per abnormal GHBT. The study concluded that older age (>55 yr) and female gender could be used as predictors of SIBO in IBS[58]. The study also showed that in subgroup of IBS subjects with bloating and flatulence as the predominant symptoms, SIBO was present in sizeable patients.

In a study by *Sachdeva et al* concluded that IBS-D subgroup, female sex & bloating as probable predictor factors of SIBO in those with IBS[19]. A study by *Law et al* concluded that hydrogen production was unaffected by PPI use on LHBT in IBS subjects (as per Rome I) [59].

Orocecal transit time (OCTT)

The importance of GI motility in the pathogenesis of gut symptoms including functional bowel diseases like IBS is well known. Various methods have been developed to assess small bowel transit time clinically. OCTT is a marker of gastric and small bowel motility time together. The techniques that are available to measure OCTT include the breath hydrogen test and gamma scintigraphy. Breath tests measure H_2 or CO_2 (which is labelled with ^{13}C or ^{14}C), which are produced when test meal undergoes metabolism by bacteria after reaching colon and these gases are produced and are exhaled. Scintigraphic tests actually measure time taken by the meal with radiolabelled marker to reach cecum.

A typical liquid meal which is used to measure OCTT contains 10 g of lactulose in 100 mL of water. Usually a solid meal to measure OCTT contains baked beans as which has stachyose and raffinose which are not absorbed in small bowel. Lactulose is a disaccharide which is not not absorbed in small bowel and gets fermented after reaching the colon by bacteria present there. It has been shown in previous studies that OCTT in healthy subjects is between 40 and 170 min for lactulose meal[60] and between 192 - 232 min for a solid meal[61]. There are multiple factors which can alter OCTT.

Studies on OCTT in IBS

Cann PA et al in1983, published a study that included 61 patients (46 females ; mean age 36 yrs) with symptoms suggestive of IBS and 53 controls. The study patients had three sub groups - constipation predominant (23), diarrhoea predominant (21) and abdominal pain/distension predominant group (17). OCTT was calculated by with solid test meal and breath hydrogen analysis. The study concluded that OCTTs were significantly shorter in subgroup of subjects with diarrhoea (3.3±0.3 vs 4.2±0.2 h; p=001) and prolonged in patients whose with constipation (5.4±0.3 vs 4.2±0.2 h; p<001) or pain and distension (5.4±0.4 vs 4.2±0.2 h; p<001) in comparison with controls[62].

In a study by *Sadik R et al* included 96 IBS and 83 controls. Among those with IBS 34 had IBS-D, 16 had IBS-C and 46 IBS-I (or A). Gastric emptying, small intestine and colonic transits were measured in all of them. At least one transit abnormality was found 51%. In

females, small intestine and colonic transit was significantly slower in IBS-C in comparison to IBS-D[63].

In a study by *Derek Yu et al* orocecal scintigraphy and LHBT both were done in 40 IBS patients (as per Rome II criteria) to assess if the rise in H2 is due to the test meal reaching the cecum. Subjects ingested a test meal which contained 99mTc and 10 g of lactulose. They all underwent LHBT and scintigrapgy at the same time. The OCTT based on scintigraphic scanning ranged from 10 to 220 min. There was a very strong correlation between the timing of cecal radioactivity positivity and rise in H2 breath ($p = 0.0025$).The mean OCTT was prolonged in IBS- as compared to IBS-D almost 2.2 times ($p =0.0023$). The authors of the study concluded that an abnormal rise in H2 measured in the LHBT can be explained by variations in OCTT and not support the diagnosis of SIBO [64].

Material and Methods

Study design

This was a prospective observational study performed in the Christian Medical College, Vellore

Inclusion criteria

- Adults aged 18 years or more who fulfilled Rome II criteria for diagnosis of Irritable Bowel Syndrome (IBS)

Exclusion criteria

- Not consenting to participate
- Recent hospitalization and/or antibiotic use in preceding 2 weeks
- Lactulose use in past one week
- Colonoscopy in past one week
- Prior major gastrointestinal surgery
- History of diabetes mellitus or other systemic illness
- Treatment history with drugs which are known to alter the gastrointestinal motility (such as anticholinergics, opiates, antidepressants, prokinetics,) or probiotics

Study setting and Population

The study was conducted in the Department of Medical Gastroenterology in CMC Hospital, Vellore from August 2012 to December 2013.Consecutive patients fulfilling inclusion and exclusion criteria were recruited from outpatient department (OPD) of the Gastroenterology clinic during the study period. The purpose of the study and methodology was explained to

these patients in detail in their own colloquial language and informed written consent was taken. The patients were scheduled to undergo glucose and lactulose hydrogen breath tests (HBTs) on two consecutive days. They were explained in detail about the procedure of the test and other instructions including diet that needed to be followed prior to test. Hydrogen breath tests were done free of cost as a part of the study protocol. No additional clinical laboratory tests were performed for the study other than those dictated for clinical care. Study plan is presented in the following flow diagram (Figure 1).

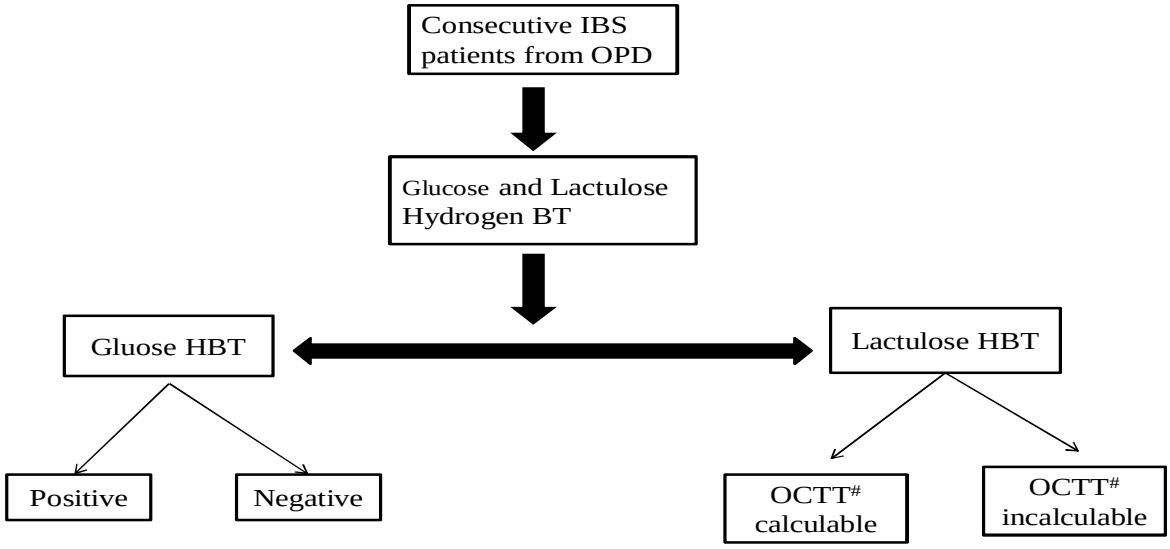


Figure 1 : Study Plan
- OCTT- Oroecal Transit Time

Informed Consent Process

Those patients who met the criteria were explained about the background and purpose of the study in detail and they were invited to participate. If the patients were willing to participate, then written consent was obtained from the patient as per consent form appended.

Ethics Committee review

The Human Ethics Committee of CMC Vellore reviewed and approved the proposal and consent forms.

Study Monitoring

The study was an investigator-initiated study conducted in Christian Medical College, Vellore. The candidate conducting this study personally ensured the entry criteria of the patient and collected the relevant clinical and laboratory details from the patient. Consecutive patients were enrolled, subject to consenting.

Methods of the Study

This study was conducted at Christian Medical College, Vellore, Tamilnadu, India. Relevant clinical and laboratory data were collected from the patients by the investigator in charge of the study, after obtaining the informed consent and ensuring entry criteria.

The patients who consented for the study underwent glucose and lactulose HBTs on two consecutive days. The following instructions were given to the patients to be followed prior coming for breath test

- To avoid non fermentable carbohydrate (pasta, breads, potato and fibre cereals) one day before the test
- Take nothing by mouth overnight (after 10 PM)
- Avoid candy and chewing gums at least an hour before the test.

- Avoid smoking and physical exercise for two hrs prior to and during testing
- To brush teeth on the morning of the day prior coming for testing

Glucose Hydrogen Breath Test (GHBT)

Breath analysis of hydrogen was done using Bedfont Hydrogen Breath Analyser, UK. Hydrogen level was measured in parts per million (ppm). Methane was not analysed in the test. Breath test was performed after overnight fast. Before the test, subjects were asked to rinse mouth with 1% chlorhexidine mouth wash to eliminate early hydrogen peak due to action of oral bacteria on test sugars. Patients gave a fasting breath for analysis of baseline hydrogen breath. This was be followed by oral intake of 70 gram of glucose dissolved in 1 glass of water (200 ml). Hydrogen in the breath was measured in ppm every 20 minutes for over next 120 min (2 hours). If there was very early peak in the breath hydrogen (basal or at 20 min), the whole test was repeated after mouth wash or postponed to next day.

Lactulose Hydrogen Breath Test (LHBT)

Subjects were asked to rinse mouth with 1% chlorhexidine mouth wash after an overnight fast. Patients gave a fasting breath for analysis of baseline breath hydrogen level. This was followed by oral intake 20 gram of lactulose in 1 glass of water (200 ml). Breath hydrogen was measured in ppm every 15 minutes over next 180 min (3 hours). Analysis of hydrogen in breath was done using Bedfont Hydrogen Breath Analyser, UK. Methane was not analysed in the test. If there was very early peak in the breath hydrogen (< 30 min), whole test was repeated after mouth wash or postponed to next day.

Sample size

Sample size was calculated according to formula

$$\mathbf{n = 4PQ/ d^2}$$

Where n = sample size, P is prevalence of the disease, Q =1-P and d is the precision

From the previous studies, prevalence of IBS was noted to be 5-25% of the general population. For calculation of sample size a mean prevalence 15 % was taken. With precision of study as 5 and prevalence of 15%, sample size was calculated as 156 from the above formula. For probable dropouts another 20 % was added and final sample size was arrived at 187.

The patients were recruited from out patients department (OPD) of Gastroenterology, Christian Medical College, Vellore who fulfilled the entry criteria and consented for the study. The following criteria were used for the study.

The Rome II diagnostic criteria for IBS

At least 12 weeks or more, which need not be consecutive, in the preceding 12 months, of abdominal discomfort or pain that has two out of three features:

(1) Relieved with defecation; and/or

(2) Onset associated with a change in frequency of stool; and or

(3) Onset associated with a change in form (appearance) of stool.

The following symptoms cumulatively support the diagnosis of IBS

- Abnormal stool frequency (for research purposes “abnormal” may be defined as greater than three bowel movements per day and less than three bowel movements per week);

- Abnormal stool form (lumpy/hard or loose/watery stool);

- Abnormal stool passage (straining, urgency, or feeling of incomplete evacuation);

- Passage of mucus;

- Bloating or feeling of abdominal distension.

* In the absence of structural or metabolic abnormalities to explain the symptoms

Patients with IBS are classified into three types using standard criteria as follows:

(i) Diarrhoea predominant (IBS-D), >3 loose stools/d

(ii) Constipation predominant (IBS-C), <3 stools/week and

(iii) Indeterminate (IBS-I), stool frequency between > 3/week and < 3/day

Positive GHBT

Positive (or abnormal) GHBT was diagnosed sustained (for at least two consecutive readings) rise of breath hydrogen by 20 ppm above basal level (if basal level was <10 ppm) or by 12 ppm above basal level (if basal level was >10 ppm). Positive GHBT was used as a surrogate marker of SIBO in the present study.

OCTT

Time interval between lactulose administration and sustained (for at least two consecutive readings) rise of breath hydrogen by 20 ppm above basal level was considered as OCTT. If

there were two peaks, second peak was taken for calculation of OCTT. Measurement of OCTT was considered to have failed if they had no peak in breath hydrogen excretion on LHBT till end of the test.

Statistical methods

The data of the present study were recorded manually into the computer and after its proper validation, checked for error; coding & decoding were compiled and analyzed using the software SPSS 15.0 for windows.

Quantitative variables were expressed as mean and standard deviation (SD) if normally distributed or median and range if not normally distributed, Qualitative variables were expressed as proportions.

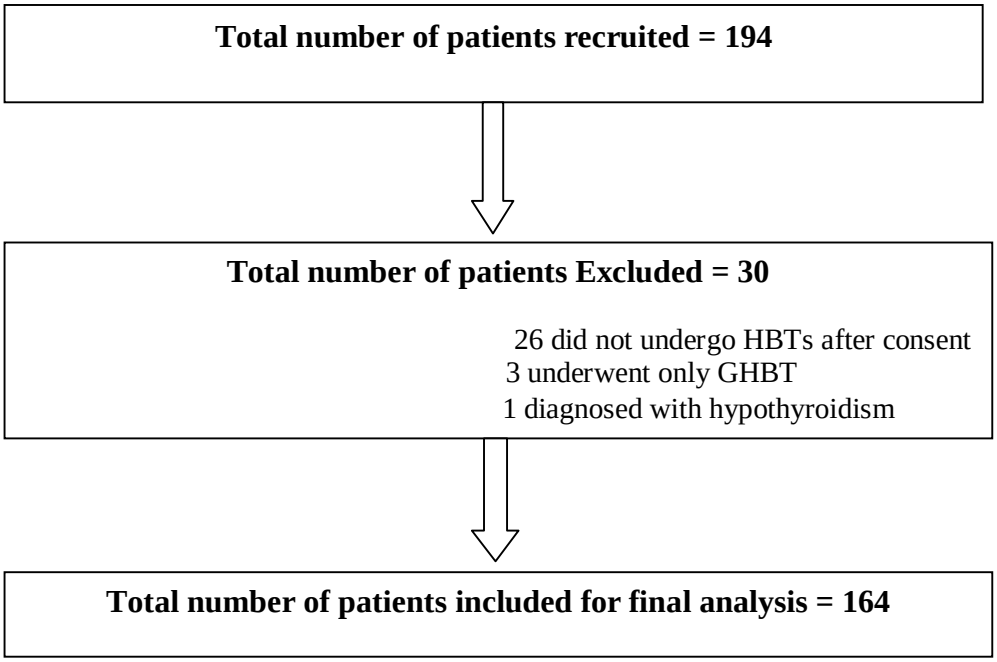
Glucose HBT was categorized as positive or negative and numbers of patients in each group correlated with additional symptoms of IBS and IBS subgroups. The analysis was done using the Chi square test or Fisher's exact test as applicable. P value of <0.05 was taken as significant.

OCTT was expressed as mean and SD in all patients together and in different subgroups of IBS. OCTT in different subgroups was analysed using analysis of variance (ANOVA) test. P value of <0.05 was taken as significant.

Results

In the present study total of 194 consecutive patients were recruited from OPD of Gastroenterology clinic. Out of these 30 patients were excluded from the study, 26 patients did not undergo breath tests despite giving informed consent, 3 patients underwent only glucose HBT and did not complete lactulose HBT, and one patient was diagnosed with hypothyroidism. After exclusion total of 164 patients were included for final analysis. This is presented in study flow chart (Figure 4).

Figure 4: Flow chart of study



Baseline characters of the patients including demographic characters and laboratory data are presented in Table no 4. Mean age of the patients was 39.16 (± 10.36) yrs. Distribution of age is depicted in figure no. 5. Majority of the patients were males comprising 68.9%, 113 out of 164 of total patients. Sex distribution is presented in figure no 6. Mean BMI of the patients was 21.78 (± 3.79) kg/m^2. Hemoglobin concentration was 13.14 (± 1.55) g/dl, with average mean corpuscular volume (MCV) of 86.39 (± 8.43) fl. Mean serum albumin level was 4.61 (± 0.36) g/dl. Average fasting blood sugar was 92.53(± 9.73) mg/dl. Mean creatinine level was1.05 (± 0.27) mg/dl in study population.

Table No. 4: Baseline characters of the patients. Some values shown are actual numbers while the others are mean ± SD

Particulars	Total No (n=164)
Age, years (mean ± SD)	39.16 (± 10.36)
Sex (Male /Female)	113/51
Height, cm (mean ± SD)	164.22 (± 7.23)
Weight, kg (mean ± SD)	59.31 (± 11.41)
BMI[#], kg/m^2 (mean ± SD)	21.78 (± 3.79)
Hemoglobin, g/dl (mean ± SD)	13.14 (± 1.55)
MCV[*], fl (mean ± SD)	86.39 (± 8.43)
TSH[$] , IU/ml (mean ± SD)	2.88 (± 2.02)
Albumin , g/dl (mean ± SD)	4.61 (± 0.36)
Fasting blood sugar, mg/dl (mean ± SD)	92.53 (± 9.73)
Creatinine, mg/dl (mean ± SD)	1.05 (± 0.27)

[#]BMI – Body mass index, [*]MCV – Mean corpuscular volume,[$]TSH – Thyroid stimulating hormone

Figure No 5: Distribution of age in study population (n=164)

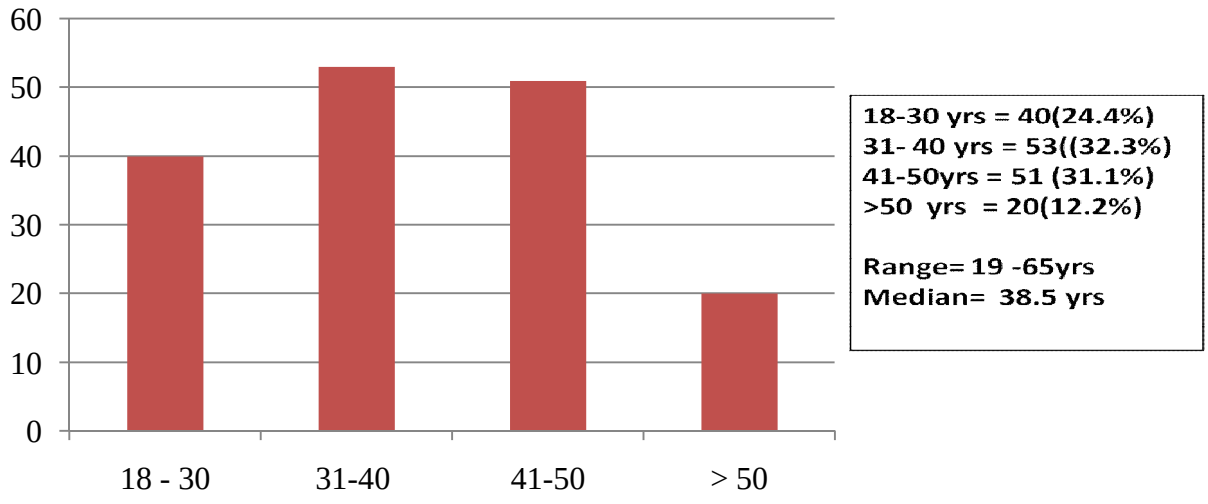
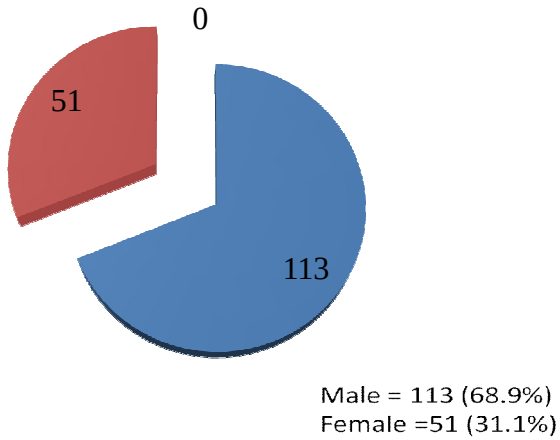


Figure No 6: Distribution of sex in study population (n=164)



Out of total 164 IBS patients, 64 (39.1%) were categorised as diarrhoea predominant (IBS-D), 24 (14.63%) as IBS –C and 76 (46.34%) were classified as IBS –I. Subtypes of IBS are presented in Figure No 7. Additional symptoms of bloating were present in 92 (56.1%), mucous in stool in 86 (52.4%) and flatulence in 29 (17.7%) of the total number of patients. Prevalence of additional symptoms of IBS is presented in Table no. 5.

Figure No 7: IBS subtype distribution (n=164)

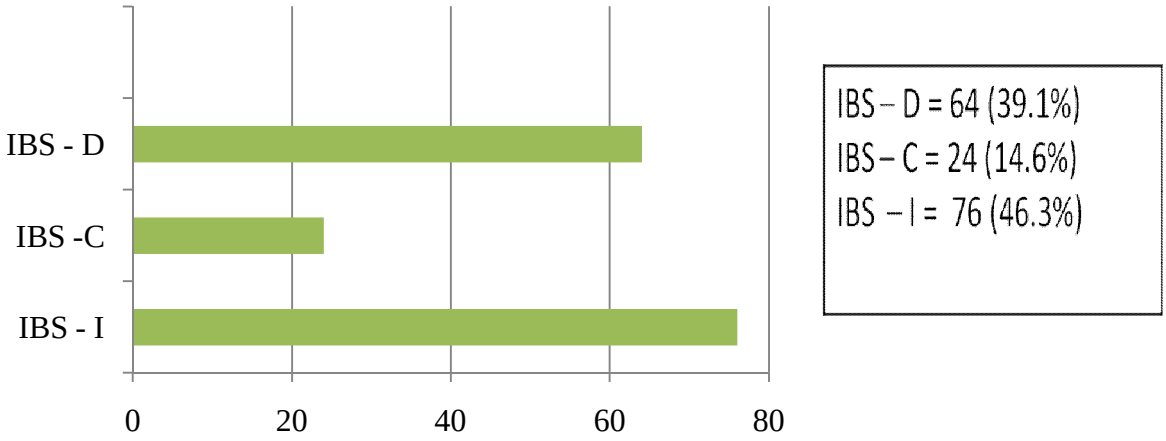


Table No. 5: Additional symptoms of IBS (n=164)

Symptom	Frequency	Percent of total patients
Bloating	92	56.1%
Flatulence	29	17.68%
Mucous in stool	86	52.44%

Baseline characters in different types of IBS are presented in table no.3 and 4. As can be inferred from table no 3, there was statistically significant (p=0.017) difference in sex distribution in different subgroups of IBS. Females were present in 45.8% (11), 36.8 % (28) and 18.8% (12) in IBS-C, IBS-I and IBS-D subgroups. Additional symptoms of IBS in different subgroups are depicted in table no 6. The symptoms of bloating (p=0.5) and flatulence (p=0.5) were not statistically different in subgroups of IBS. Mucous in the stool was statistically different in subgroups of IBS with p value of 0.05, with symptom present in 64.1% (41) in IBS-D, 50 % (12) in IBS –C and 43.4 % (33) in IBS-I.

Table no 6: Distribution of sex in IBS subgroups

IBS subgroup	Sex		Total	P value
	Male	Female		
IBS-D (%within IBS subgroup)	52 (81.3%)	12 (18.8%)	64	0.017
IBS-C (%within IBS subgroup)	13 (54.2%)	11 (45.8%)	24	
IBS-I (%within IBS subgroup)	48 (63.2%)	28 (36.8%)	76	
Total	113 (68.9%)	51(31.1%)	164	

P value as per Pearson's chi square test

Table no. 7: Baseline characters in different subgroups of IBS

IBSTYPE		Age (years)	BMI (kg/m^2)	HB (g/dl)	MCV (fl)	Albumin (g/dl)	TSH (IU/ml)	FBS (mg/dl)	Creatinine (mg/dl)
IBS-D (n=64)	Mean	38.88	21.57	13.36	87.09	4.64	2.93	91.72	1.11
	SD	10.62	3.82	1.7	8.62	0.45	1.79	9.38	0.37
IBS-C (n=24)	Mean	40.54	20.83	12.41	87.16	4.51	3.05	90.08	1.01
	SD	10.57	3.55	1.55	8.5	0.29	1.97	10.07	0.14
IBS-I (n=76)	Mean	38.96	22.262	13.19	85.57	4.62	2.77	94.00	1.02
	SD	10.167	3.8067	1.35	8.29	0.29	2.23	9.80	0.19
Total (n=164)	Mean	39.16	21.784	13.14	86.39	4.61	2.87	92.53	1.05
	SD	10.359	3.7886	1.55	8.42	0.36	2.02	9.73	0.27

Table no. 8: Distribution of additional symptoms of IBS in subgroups

		Bloating		Flatulence		Mucous in stool	
		Yes	No	Yes	No	Yes	No
IBS TYPE	IBS-D	33	31	11	53	41	23
	IBS-C	15	9	6	18	12	12
	IBS-I	44	32	12	64	33	43
Total		92	72	29	135	86	78
P value		0.5		0.5		0.05	

P value as per Pearson's chi square test

Glucose HBT was abnormal or positive in 12 patients out of total 164 patients who underwent test. Prevalence of abnormal or positive GHBT, which is a surrogate marker of SIBO, was 7.32%. Among those who had abnormal GHBT, 7 were in IBS-D subgroup, 4 in IBS-I and one in IBS-C subgroup. On subgroup analysis prevalence of abnormal GHBT was 10.4% (7/64) in IBS-D, 5.3 %(4/5.3%) and 4.2% (1/24) in IBS-C patients. This data is presented in figure no. 8 and table no 9.

Figure No. 8: Positive glucose hydrogen breath test (GHBT)

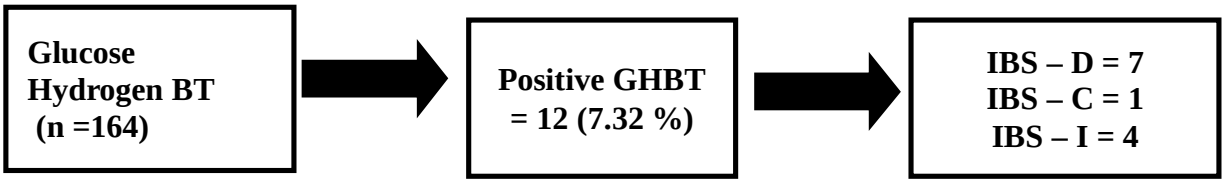


Table No. 9: Positive glucose hydrogen breath test (GHBT)

	Positive GHBT	Total no of patients	Percent prevalence
All IBS patients	12	164	7.32%
IBS - D	7	64	10.4%
IBS - I	4	76	5.3%
IBS-C	1	24	4.2%

Positive GHBT: correlation with sex, age and IBS type

In order to assess if any predictors can be found for positive GHBT (surrogate marker of SIBO) various factors were analyzed. Positive GHBT was not statistically different in males or females (p = 0.518, Fisher's exact test) which is presented in table no 10. Another parameter which was shown to be significantly associated with SIBO, age >55 was assessed. As can be seen from table no. 11, age >55 years was not significantly associated with positive GHBT (p=0.97, Fisher exact test).

Table no. 10: Correlation of positive GHBT with sex

		SEX		p value*
		Male	Female	
GLUCOSE HBT	Positive	7	5	0.518
	Negative	106	46	

*p value using Fisher's exact test

Table no.11: Correlation of positive GHBT with age

		Age		p value*
		age<55	Age>/=55	
GLUCOSE HBT	Positive	9	3	0.97
	Negativ	139	13	

*p value using Fisher's exact test

IBS subtype was analyzed to see whether correlation existed with positive GHBT. As can be seen from table no. 12, IBS subtype was not statistically associated with positive GHBT (p=0.67, Pearson's chi square test).

Table no. 12: Correlation of positive GHBT with IBS subtype

		IBS subgroup			p value *
		IBS-D	IBS-C	IBS-I	
GLUCOSE HBT	Positive	6	1	5	0.67
	Negative	58	23	71	

*p value using Pearson's chi square test

Correlation with additional symptoms of IBS with positive GHBT

Additional symptoms of bloating, flatulence and mucous in stool were correlated with positive glucose HBT to see if any symptom is positively correlated. Bloating was correlated with positive GHBT which was statistically significant with p value of 0.013 using chi square test. The other additional symptoms of flatulence and mucous in stool were not statistically associated with positive GHBT, with p value of 1.00 and 0.769 respectively. This is depicted in table no 13.

Table no 13. Correlation with additional symptoms of IBS

		GLUCOSE HBT				
		Positive		Negative		p-value
		n	%	n	%	
Bloating	Present	11	91.67	81	53.29	0.013
	Absent	1	8.33	71	46.71	
Flatulence	Present	2	16.67	27	17.76	1.00
	Absent	10	83.33	125	82.24	
Mucous in stool	Present	7	58.33	79	51.97	0.769
	Absent	5	41.67	73	48.03	

p value with Fisher's exact test

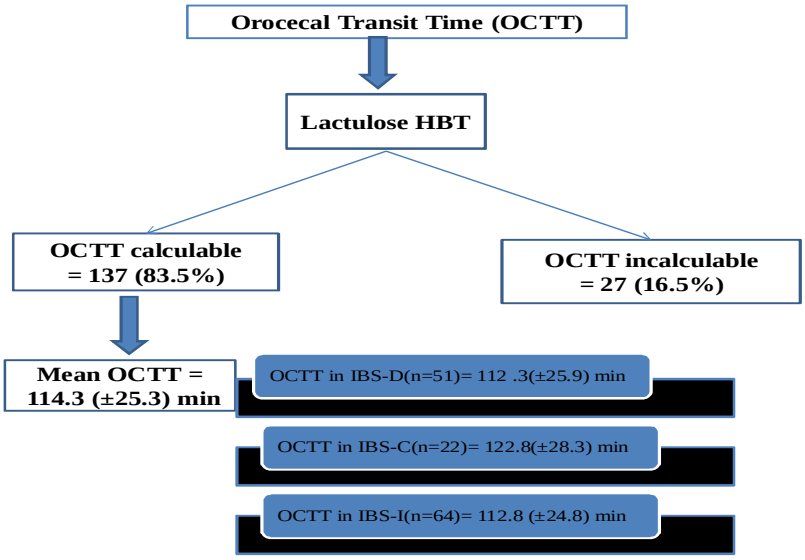
Orocecal Transit Time (OCTT)

OCTT was calculated from lactulose hydrogen breath test (LHBT) in the study. Out of total 164 patients, OCTT could be calculated in 137(83.5%) and incalculable in 27 (16.5%) patients. Among 27 patients where OCTT was incalculable, 13 were in IBS-D subgroup (13/64, 20.3%), 12 in IBS-I (12/76, 15.8%) and 2 in IBS-C (2/24, 8.3%) subgroups. This is depicted in table no. 14. Mean OCTT in all patients where it could be calculable (n=137) was114.3 (±25.3) minutes. Mean OCTT in IBS-D, IBS-C and IBS-I was 112.3 (±25.9) min, 122.8 (±28.3) min and 112.8 (±24.8) min respectively. The data on OCTT is presented in figure no. 9.

Table no 14. Patients with calculable/Incalculable OCTT in subgroups of IBS

IBS subgroup	OCTT calculable	OCTT incalculable	Total No	Percentage of incalculable OCTT
IBS - D	51	13	64	20.3%
IBS - C	22	2	24	8.3%
IBS - I	64	12	76	15.8%
Total	137	27	164	16.5%

Figure no. 9: Orocecal Transit Time (OCTT)



Comparison of OCTT in different subgroups of IBS

Among the patients in whom OCTT is calculable, it was compared within subgroups of IBS to find out if OCTT was different statistically. When analyzed all three subgroups together using Analysis of Variaence (ANOVA) test, there was no statistical difference in OCTT among these subgroups with p value of 0.255. This is depicted in figure no 10 and table no. 15.

Figure no 10: Comparison of OCTT in different subgroups of IBS

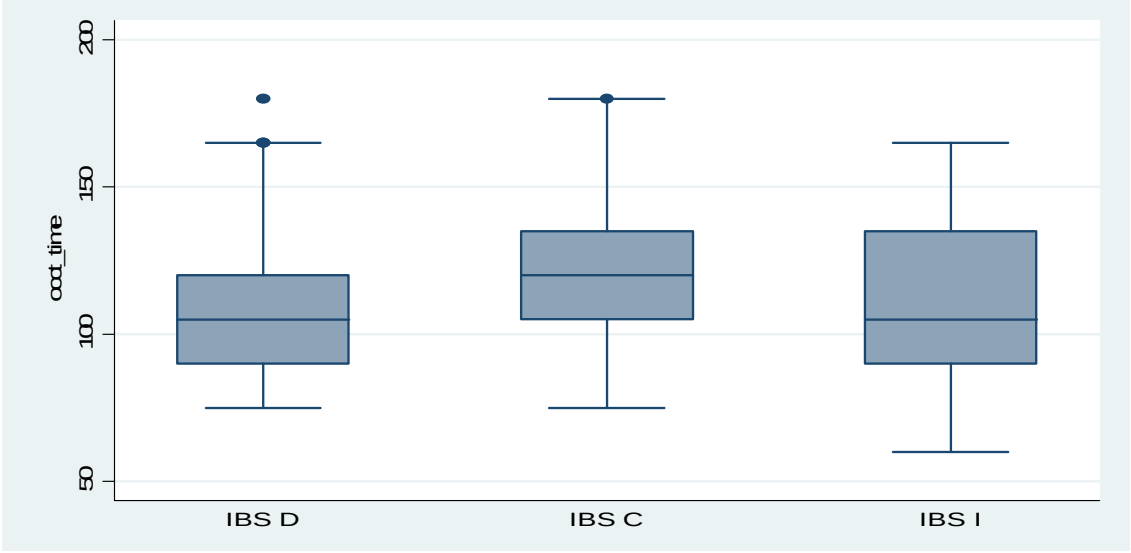


Table no 15: Comparison of OCTT in different subgroups of IBS

IBS subgroup	n	Mean	Standard Deviation	p-value*
IBS D	51	112.34	25.96	0.255
IBS C	22	122.86	28.31	
IBS I	64	112.86	24.82	

*p value as per ANOVA test

The post hoc tests done also reveled there is no difference between the IBS-D and IBS C as well as the other combinations.

Correlation of OCTT with positive GHBT

Mean OCTT in 12 patients of IBS with positive GHBT was 113.75 (±30.31) min and in those 121 patients with negative GHBT was 114.3 (± 25.34) min. OCTT was not statistically different (p=0.94, t test for equality of means) between the two groups.

Discussion

IBS is a common functional bowel disease with high prevalence in general population. Despite its high prevalence in general populations and the health care costs involved in management, the aetiology of IBS is not known exactly. A number of different mechanisms have been implicated in the pathogenesis of IBS[11,12]. One of the associations of IBS which has been proposed is with SIBO and has varied association in various studies done across the globe. Few of previous studies have shown that colonic and small bowel transit is prolonged in IBS-C and accelerated in IBS-D[8].

The present study was planned to find out prevalence of SIBO (as assessed by abnormal Glucose HBT) in IBS subjects and also to see whether it correlated with symptoms. Another objective of the study was to assess oro-cecal transit time (OCTT) in these subjects (using lactulose HBT) and to see if any difference existed in transit time of IBS subgroups. The total number of patients included for final analysis of study was 164.

Mean age of the patients in our study was 39.16 (±10.36) years. Age group ranged between 18-65 years. Among them 24.4% (40) were in age group of 18-30 yrs, 32.3% (53) in 31-40, 31.1% (51) in 41-50 and 12.2% (20) patients in >50 years. Hence majority of the patients were middle aged. In a community based study of IBS patients by *Shah et al* the mean age was 37.2 years[6]. In a prospective study by *Makharia et al* the mean age was 34.6 (± 10.8) years[25].

In our study majority of patients were males comprising, 68.9% (113) and females comprised 31.1% (51). This is similar to other hospital based studies from India which evaluated SIBO

in IBS, 71.1% (160/225) in *Rana SV et al*[17], 69.5% (41/59) by *Sachdeva et al*[19]. But in population based studies from India have shown varied sex distribution. In a community based study of IBS by *Shah et al* showed slight male predominance with 56% (1441/2549). In study by *Makharia et al*, prevalence of IBS was higher in females when compared with males 4.8% vs 3.2%[25]. Studies from the west have always shown IBS being female predominant disease both in hospital and community based studies with Male to female ratio being 1:2 [8,15].

Baseline characters of the study are presented in table no. 4 including routine investigations. As can be viewed from the table most of the reports are within normal ranges implying that patients did not suffer from organic diseases.

In our study IBS-I was the predominant subgroup with 46.3% (76) followed by IBS-D in 39.1% (64) and IBS-C in 14.6% (24). Males and females were significantly different in these subgroups of IBS with p value of 0.017 (table no 6). Females comprised 45.8% (11/24), 36.8% (28/76) and 18.8% (12/64) in IBS –C, IBS-I and IBS-D respectively. Baseline characters were almost similar in different subgroups of IBS (table no. 7). In study by *Makharia et al* 4767 IBS (as per Rome III) subjects from rural north India, included subgroups of IBS as - IBS-M in 42.4%, IBS-D 37.7%, IBS-U 13.6% and IBS-C in 6.3%. As compared with this population based study, our study also showed indeterminate or mixed was most common and constipation predominant was least common.

Additional symptoms of bloating, flatulence and mucous in stool were present in 56.1% (92), 17.68% (29) and 52.44% (86) respectively among total of 164 patients. Bloating and

flatulence were similar in different subgroups of IBS (p value of 0.5 each, table no. 8). But additional symptom of mucous in stool was significantly different in these subgroups of IBS with p value of 0.05.

In the present study Positive (or abnormal) GHBT was noted in 7.32% (12/164). This test is used as a surrogate marker of SIBO in the present study. Hence prevalence of SIBO in the study was 7.32%. Among these 12 patients with positive GHBT, 7 (10.4%) were in IBS-D, 4 (5.3%) in IBS-I and 1 (4.2%) in IBS-C.

In a study of 202 (as per Rome I) IBS patients by *Pimentel et al* using LHBT results suggesting SIBO was found in 157 (78%) of patients. LHBT was done with 10 g of lactulose and positive HBT taken as >20 ppm above baseline[15]. In another study by the same group with same study design, abnormal LHBT was present in 84% (93/111) vs 20% (3/15) in the subjects with IBS and healthy subjects respectively[16]. In a study from Italy, positive GHBT was found in 31% (65) of IBS (as per Rome II), patients as compared to 4% (102) in the control group which was significant difference[51]. In this study glucose HBT was done with 50 g and positive HBT taken as >10 ppm above baseline. A study by *Rana SV* et al. from PGI, Chandigarh showed that the prevalence of SIBO was 11.1% (225) in IBS subjects as compared to 1% in controls (100). The study used GHBT (50 gm glucose) to diagnosed SIBO, and positive HBT was taken as >12 ppm above baseline[17]. Prevalence of SIBO in IBS (Manning criteria) was 8.5%. in a study by *Ghoshal UC.* In this study rise in H2 above 12 ppm from baseline after 100 g glucose was taken as positive for SIBO[18]. SIBO (i.e. positive GHBT) in patients with IBS (Rome III) was 23.7% (14/59) in study by *Sachdeva et al.* Both

last two studies used rise in H2 above 12 ppm from baseline after 100 g glucose was taken as positive for SIBO[18].

Our study showed the lowest prevalence of SIBO (positive GHBT) including other studies from north India. Possible reasons for low incidence include

(a) We did not analyze methane in the study which might have led to under estimation of positive GHBT in those who are predominant methane and hydrogen non producers

(b) Use of stringent criteria for positive (or abnormal) GHBT. In our study Positive (or abnormal) GHBT was diagnosed sustained (for at least 2 consecutive readings) rise of breath hydrogen by 20 ppm above basal level (if basal level was <10 ppm) or by 12 ppm above basal level (if basal level was >10 ppm). We postponed the HBT if baseline breath hydrogen was high.

Other possible reasons of variation in dose of glucose used, we used 70 gram, dose of which varied in different studies.

We analysed the different factors to see if any correlation existed with positive GHBT. Additional symptom of bloating was correlated with positive GHBT with statistical significance (p value 0.013, Fisher's exact test). Additional symptoms of flatulence (p=1.0), mucous in stool (p=0.76), IBS subtype (p=0.67), sex (p=0.518) and age >55 (p=0.97) did not correlative significantly with positive GHBT. In a retrospective study *Reddymasu et al*, which included 98 IBS patients (Rome II) with predominant symptoms of bloating & flatulence, showed that older age (more than 55 yr) and female gender were predictors of

SIBO in subjects with IBS[58]. A study by *Sachdeva et al* concluded that IBS-D subgroup, female sex and bloating as possible predictors [19]. A study by *Law et al* concluded that PPI therapy did not affect H2 production on LHBT in patients with IBS [59].

With this we can postulate that if IBS patients with bloating undergo GHBT, it is likely SIBO may be diagnosed more frequently. Path physiologically bacterial overgrowth in small bowel by gas forming organisms can lead to excessive gaseous distension of abdomen which could explain symptom of bloating in such patients.

In the present study OCTT was calculated from lactulose hydrogen breath test (LHBT). Out of total 164 patients, OCTT could be calculated in 137(83.5%) and incalculable in 27 (16.5%) patients. OCTT was called incalculable if there was no rise in breath hydrogen level till the end of LHBT i.e. 180 min. Mean OCTT in all patients where it could be calculable (n=137) was114.3 (±25.3) minutes. Mean OCTT in IBS-D, IBS-C and IBS-I was 112.3 (±25.9) min, 122.8 (±28.3) min and 112.8 (±24.8) min respectively. Two previous studies have shown that OCTT in healthy population is between 40 and 170 min for a liquid meal containing lactulose [60] and between 192 - 232 min for a solid meal[61]

The study showed that OCTT was incalculable in 27 (16.5%) patients. It is possible that these patients are hydrogen non producers and predominant methane producers instead. Approximately 15% of the healthy subjects are considered as predominant methane producers. These indivisual's intestines are colonized with *Methanobrevibacter smithii*. In these persons hydrogen gas produced reacts biochemically with carbon dioxide to produce

methane. Thus they produce less hydrogen in breath. This may have affected OCTT results of ours study. But these patients (where OCTT was incalculable) were present in all subgroups of IBS patients, 13 were in IBS-D subgroup (13/64, 20.3%), 12 in IBS-I (12/76, 15.8%) and 2 in IBS-C (2/24, 8.3%) subgroups.

There was no statistical difference in OCTT among three subgroups of IBS when analyzed with ANOVA with p value of 0.255. Post hoc tests also did not show any difference in OCTT among two subgroups of IBS together. A study *Cann PA et al* included 61 patients with IBS and 53 controls. The study concluded that OCTTs were significantly shorter in subgroup of subjects with diarrhoea (3.3±0.3 vs 4.2±0.2 h; p=001) and prolonged in patients whose with constipation (5.4±0.3 vs 4.2±0.2 h; p<001) or pain and distension (5.4±0.4 vs 4.2±0.2 h; p<001) in comparison with controls[62]. In a study by *Sadik R et al* that included 96 IBS subjects showed that small intestine and colonic transit was prolonged in IBS-C in comparison with IBS-D subgroup[63]. *Yu et al* in a study showed that the mean OCTT was prolonged in IBS-C as compared to IBS-D almost 2.2 times [64].

Limitations and strengths of the study

There were many limitations in our study. Firstly, we did not analyze methane in the breath which may have caused underestimated the prevalence of SIBO and affected OCTT result as it was incalculable in 16.5%. Secondly, there was no control population for comparison. Strengths of our study include good sample size which included all subtypes of IBS and stringent criteria used for abnormal GHBT.

Summary and Conclusions

- IBS-I was the most common and IBS-C the least common subgroup of IBS
- Males predominated among the all IBS patients, but sex distribution was significantly different in subgroups
- Prevalence of abnormal glucose hydrogen breath test (a surrogate marker of SIBO) was 7.32% which is lower than described in studies from North India
- The symptom of bloating was significantly correlated with positive GHBT
- IBS subtype, age or sex did not correlate with positive GHBT
- Orocecal transit time (OCTT) was incalculable in 16.5% which may represent hydrogen non producers
- Mean OCTT was114.3 (±25.3) minutes in those with calculable OCTT
- Orocecal transit time was not different in subgroups of IBS

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APPENDIX

STUDY NUMBER:

PROFORMA FOR BREATH TEST IN IBS

Name	
Hospital No	
Age	
Sex	
Occupation	
Residence	
Telephone	**Local :** **Permanent:**

Smoking : **Yes /No** **If Yes, Details:**

Alcohol : **Yes/No** **If Yes, Details:**

SYMPTOMS/DETAILS:

Pain Abdomen	**Yes/No**	**Upper/Lower/Generalized**	
Abdominal Discomfort	**Yes/No**		
Duration 12 weeks or more in last 12 months	**Yes/No**		

Essential Symptoms of pain/discomfort

Relived by defecation	**Yes/No**	
Associated with change in Stool Frequency	**Yes/No**	
Associated with change in Stool Consistency	**Yes/No**	

Additional Symptoms

Abnormal stool Frequency	**Yes/No**	**<3/wk : >3/d : None of these**	
Abnormal Stool Form	**Yes/No**	**Hard/Loose**	
Abnormal Stool Passage	**Yes/No**	**Straining/Urgency/Incomplete evacuation**	
Passage of Mucous	**Yes/No**		
Bloating or Distension of abdomen	**Yes/No**		
Others : Flatulence	**Yes/No**		

HT: _____ cms **WT:_____ kg** **BMI _____ kg/m^2**

SIGNS – Any Significant

LABS AND OTHER INVESTIGATIONS

BLOOD:

Hb	**MCV**	**A/G**	**TSH**
AC	**Creatinine**		
Others			

ENDOSCOPY

IMAGING STUDIES

IBS Subtype: Diarrhoea Predominant ( )
Constipation Predominant ( )
Indeterminate ( )

GLUCOSE HYDROGEN BREATH TEST - Positive/Negative

0 MIN	**20**	**40**	**60**	**80**	**100**	**120**

LACTULOSE HYDROGEN BREATH TEST

0 MIN	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**	**135**	**150**	**165**	**180**

Orocecal Transit Time (OCTT) - Calculable / Incalculable

OCTT - _____ min

MASTER CHART

STUDY NO	HOSPITAL NO	AGE	SEX	RESIDENCE	IBS TYPE	BLOATING	FLATULENCE	MUCOUS	HEIGHT	WEIGHT	BMI	GLUCOSE HBT	LACTULOSE HBT	OCTT CALCULATION	OCTT TIME	HB	MCV	TP	ALBUMIN	TSH	AC	CREATININE
1	623846D	42	1	2	3	1	2	2	160	45	17.6	2	2	2		13.9	94.3	7.6	4.8	0.33	115	0.5
2	287509F	41	1	1	3	2	2	2	162	53	20.2	2	2	2		14.7	88.4	8.6	5.3	4.6	94	0.9
3	101871C	63	1	2	1	1	2	1	160	53	20.7	2	2	1	105	13.8	76	7.4	4.5	1.36	86	0.8
4	837690D	57	2	1	3	2	2	1	150	53	23.6	2	2	2		12.3		7.4	4.4	7.5	109	1.1
5	344604F	39	1	5	1	2	2	1	162	70	26.7	2	2	1	120	13.8	84.3	8	4.9	3.2	119	1.2
6	344759F	47	1	1	3	2	2	1	166	55	20	2	2	2		12.7	67.4	8.4	4.6	4.7	89	1.3
7	827474D	37	1	5	3	1	1	2	175	74	24.2	2	2	1	105	13.3	75	7.3	4.7		97	1.1
8	390561F	25	1	1	3	2	2	2	172	84	28.4	2	2	1	135	15.7	82.6	8.3	5.2	0.73	78	0.4
9	377895F	35	1	6	1	2	2	1	161	52	20.1	2	2	2		14.7	86.4	8.4	5.7	2.7	76	0.9
10	353722F	43	1	5	3	2	2	2	171	67	22.9	2	2	1	135	13.9	72.6	8.6	4.9	2.5	107	1.13
11	352527F	61	1	1	2	1	1	1	165	48	17.6	2	2	1	120	13.5		7.5	4.8	2.66	85	1.09
12	357856F	43	1	5	1	2	2	2	170	65	22.5	2	2	1	105	13.5	92.7	7.5	4.8	2.69	93	1.18
13	388279F	40	2	1	2	1	2	1	149	43	19.4	2	2	1	120	9.8	89.2	7.7	4.4	2.8	88	1.13
14	380118F	38	1	1	2	2	2	1	169	58	20.3	2	2	1	120	13.7	91.4	7.9	4.6	0.97	87	1.2
15	404342F	21	1	1	1	2	2	1	176	52	16.8	2	2	2		14.2	90.6	7.3	4.8	1.36	77	0.91
16	404915F	51	1	1	2	1	2	1	172	50	16.9	2	2	1	105	13.5	91.8	7.2	4.2	2.82	68	1.26

17	173104F	46	1	1	3	1	2	2	168	65	23	2	2	1	120	11.5	67.7	7.4	4.8	3.2	93	0.96
18	405454F	50	1	6	1	2	2	1	172	60	20.3	2	1	1	105	13.1		7.6	4.5	3.8	92	1.23
19	416004F	31	2	1	1	1	2	1	151	52	22.8	2	2	1	120	12.1	81.3	6.7	4	9.03	92	0.94
20	219085F	31	1	3	1	1	2	1	164	60	22.3	2	1	1	120	17	80	7.9	5	2.98	78	1.2
21	410490F	45	2	1	2	1	2	2	160	55	21.5	2	2	1	75	13	91.2	7.7	4.8	2.1	102	1.05
22	357634F	37	1	1	3	2	2	1	150	68	30.2	2	2	1	90	12	86.7	7.6	5.1	3.4	94	1.31
23	419435F	26	1	5	3	2	1	2	168	48	17	2	2	1	105	12.8	88.9	8.4	4.4		94	1.1
24	373442F	29	1	1	3	1	2	1	161	60	23.1	2	2	1	75	13.8	83.7	7.6	4.7	13.8	98	1.1
25	372011F	43	1	1	1	2	2	1	170	57	19.7	2	1	1	105	13.7	95.6	7.4	4.7	0.9	100	1.2
26	414457F	32	1	6	1	1	2	1	164	47	17.5	2	2	1	150	14.9	88.3	7	4.5	2.3	89	1.12
27	369150D	52	1	5	1	1	1	1	174	74	23.8	2	2	2		14.4	104	7.4	4.3	3.7	92	1.19
28	362366F	48	2	1	1	1	2	2	149	63	28.4	2	1	1	120	11.7	91	7.3	4.3		104	0.96
29	408070F	23	1	5	1	1	2	2	165	70	25.7	2	2	1		14.3	111	7.2	4.9	5.15	90	1.14
30	362368F	24	2	1	3	1	2	2	157	60	24.3	2	1	1	120	10.1	79	7.1	4	3.13	85	0.9
31	405955F	45	1	1	1	1	2	2	169	51	17.9	2	2	1	150	15.1	97	6.8	4.4	2.44	102	0.99
32	388423F	26	1	1	1	2	2	1	178	56	17.7	2	1	1	90	14.2	87.8	7.4	4.8	2.8	91	1.04
33	419707F	45	2	2	1	1	2	2	157	56	22.7	2	2	1	135	9.1	85.5	8.3	4.5	7.7	108	0.83
34	419710F	63	2	2	1	2	2	2	159	73	28.9	1	1	1	90	10.2	81.5	8	4.4	4.5	97	0.96
35	366393	30	2	1	2	2	2	1	155	65	27	2	1	1	12	13	90	7.	4.7	9.	98	0.97

	F										.1				0	.2	.4	5		8		
36	414925F	39	1	1	3	1	2	1	165	60	22	2	2	2		15	78	7.5	4.8	1.05	85	1.06
37	397062F	21	1	1	1	2	2	1	167	56	20.1	2	2	1		17.1		8.7	5	0.76	85	0.98
38	408491F	33	2	1	3	2	2	1	149	55	24.8	2	2	1	165	12.6	98.7	7.5	4.6	10.4	101	0.94
39	416504F	37	1	1	3	2	2	1	173	55	18.4	2	2	1	135	13.8	89.7	8	4.7	3.12	91	1.31
40	413166F	65	2	1	3	1	2	2	161	45	17.4	1	2	1	150	12.1	86.4	7.7	4.4	2.07	102	1.02
41	362668F	28	1	5	3	2	2	2	170	61	21.1	2	1	1	105	14.7	100	7	4.8	1.59	94	1.32
42	407333F	45	1	1	1	2	2	1	171	64	21.9	2	2	1	180	14.6	95.6	7.4	4.9	2.84	93	1.18
43	829570	36	1	3	3	2	2	2	166	65	21.9	2	1	1	105	13		7.4	4.9			1.2
44	427521F	58	1	5	1	2	2	1	168	60	21.3	2	2	2		13.9	60	7.3	4.7	3.69	94	3.69
45	752513D	37	2	1	2	2	2	1	148	50	22.8	2	2	1	135	11.2	94	7.9	4.7	3	90	1
46	433492F	25	1	1	1	1	2	2	162	50	19.1	1	1	1	120	16.3	84.1	8	4.8	1.4	88	1.29
47	433908F	48	2	5	1	1	2	1	158	65	24.9	2	2	1	105	11.5	91.4	7.7	4.6	4.8	94	0.69
48	383550F	28	2	3	2	2	2	2	154	45	19	2	1	1	120	12.5	83.7	7.5	4	0.97	81	0.98
49	290950F	33	1	1	1	1	2	1	164	60	22.6	1	1	1	90	13	84.4	7	4.5	2.3	83	1.04
50	261297F	30	1	1	3	1	2	1	165	69	25.3	2	2	1	135	11.7	63.1	7.6	4.7	3.5	93	1.1
51	243680F	35	2	1	1	2	2	2	158	58	23.2	2	2	2		10.9	83	7.3	4.6	4.5	82	1
52	283751F	55	1	2	2	2	1	2	170	68	23.5	2	2	1	165	10.9	92.2	7.6	4.6	2.5	88	0.91
53	251727D	44	1	5	1	2	2	2	158	65	25.9	2	1	1	90	13.4		6.9	4.7	2.2	97	1.1

54	984396B	31	1	2	1	2	2	1	163	44	16.6	2	2	1	135	13.3	90.8	7.7	5.1	2.32	94	1.07
55	452645F	45	1	6	1	2	1	1	165	50	18.4	2	2	1	105	13.2	88.7	7.3	4.2	1.74	99	0.88
56	199995B	55	1	3	1	2	2	2	168	65	23	2	2	1	120	13	76	7.2	4.7	1.5	85	1
57	285208B	41	1	3	1	1	2	2	174	94	30.6	2	2	1	150	14.8	92.8	7.4	4.6	4.5	99	1.51
58	408475B	47	2	1	1	1	2	2	156	50	20.5	2	2	2		12.1	88.6	7.4	4.5	7.2	114	1.04
59	305586F	19	2	1	3	2	1	2	165	50	18.9	2	2	1	120	11.4	71.9	8.9	4.7	0.61	83	0.78
60	466592F	50	1	1	1	2	1	2	166	52	18.9	2	2	1	105	12.5	86.1	6.8	4.5	1.51	104	1.37
61	477561F	27	1	3	1	1	2	2	172	74	25	2	2	1	105	13.2	84	7.2	4.8		64	1.17
62	326693F	48	2	1	1	1	2	2	156	54	27.2	2	1	1	90	8.8	66.9	8.1	1.95	1.95	90	1.36
63	709291D	40	2	1	3	2	2	1	150	55	24.4	2	2	1	135	12.2	84.6	6.8	4.4	3.75	74	0.85
64	811112D	46	1	1	1	2	2	1	165	45	16.5	2	2	1		12.3	86.5	8.1	4.7	7.2	87	1.12
65	782675D	38	1	1	3	1	2	2	174	80	26.4	2	2	1	135	14.1	87.5	8	3.9	1.399	103	1.13
66	588538D	40	1	1	1	2	2	1	165	45	16.5	2	2	1	165	15.2	89.7	7.3	4.6	2.07	92	1.14
67	393985F	41	1	3	3	2	2	2	168	74	26.2	2	2	1	105	13.5	88.2	6.9	4.4	1.91	103	1.1
68	233580F	59	1	1	1	1	2	2	174	63	20.8	2	2	1	105	11.9	90.9	7.5	4.3	3.09	95	1.24
69	473466F	31	1	5	3	1	2	2	178	65	20.5	2	2	1	120	14.5	103	7.5	4.6	3.5	90	1.15
70	039096D	47	1	1	3	1	2	2	174	67	20.4	2	2	1	135	13.7	94.4	7	4.6	1.6	104	1.13
71	080570D	59	1	2	3	2	2	2	168	64	23	2	2	1	120	12.4	86.4	7.2	4.3	2.18	101	1.38
72	559578	35	1	1	1	2	2	2	164	60	22	2	2	1	90	13	93	6.	4.5	2.	98	1.15

	C										.3					.8	.4	8		71		
73	009215F	50	1	1	3	2	2	1	170	48	16.6	2	2	1	105	13.5	93.4	7.2	4.5	3.98	84	1.1
74	118197F	43	2	1	2	1	2	2	160	54	21.1	2	2	1	90	11.8	86.2	6.7	3.8	3.6	93	0.9
75	456958D	26	2	1	3	1	2	2	155	56	23.3	2	2	1	90	12.5	91	7.9	5	2.08	99	0.82
76	483108F	38	2	1	1	1	2	1	150	50	22.1	2	2	2		9.5	89.1	7.2	4.5	0.9	104	0.85
77	571528D	59	2	1	1	2	2	1	151	55	24.1	2	2	1	105	11.3	89	7.4	4.2	2.2	98	1.11
78	762306C	38	1	5	3	1	1	2	178	76	24	2	2	1	75	13.8	78	7.4	4.2	2.57	88	1.12
79	342129f	41	1	1	3	2	2	1	171	60	23.3	2	2	1	105	14	86	8.2	4.7	5.1	110	1.28
80	424762f	35	1	6	1	1	1	1	162	58	22.1	1	1	1	90	13.4	74	7.6	4.4	0.44	73	0.8
81	685377D	53	2	1	3	1	2	1	154	50	21.1	2	2	2		14.3	91	7.9	4.7	3.09	107	0.94
82	362401D	49	1	2	1	1	2	2	162	63	24	2	2	1	75	14.1	88.8	7.4	4.7	6.4	96	1.14
83	413034F	23	1	1	3	2	2	2	170	64	22.1	2	2	1	90	13.8	78	7.1	4.8	2.67	80	1.16
84	298921D	30	1	3	3	1	2	2	160	48	18.7	2	2	1	105	15.2	89.4	8	4.5	1.29	71	1.04
85	902496D	49	2	2	3	1	2	1	158	61	24.4	1	2	1	90	10.4	70.5	7.3	4.3	2.7	96	0.98
86	136740F	35	1	1	2	1	2	1	165	45	16.5	2	2	1	180	14.2	92.4	8.1	4.8	2.1	87	0.8
87	613404F	20	1	5	1	1	2	1	170	61	21.1	2	2	1	75	15.3	87.2	7.3	5	1.45	80	0.9
88	675106D	40	1	1	1	2	2	1	160	58	22.7	2	2	2		14.6	83.5	7.7	4.6	3.2	95	1.15
89	551123D	29	1	2	2	2	2	1	170	56	19.4	2	2	1	105	15.4	95.4	7.3	4.8	1.78	76	1.09
90	958575D	46	1	5	3	1	2	1	170	98	33.9	2	2	2		14.8	95	7.9	4.4	2.04	85	1.49

91	409509F	39	2	1	3	2	2	2	161	57	22	2	2	1	120	12	91.4	7.3	4.7	1.07	104	0.79
92	399140F	31	2	1	1	1	2	1	171	55	18.8	2	2	1	75	12.3	92.6	6.9	4.7	1.4	83	0.99
93	610632F	31	1	6	1	1	2	1	161	58	21.2	2	2	1	165	14.5	90.9	7.7	4.9	1.63	88	0.88
94	442562F	38	1	3	3	1	2	2	171	80	27.4	2	2	1	135	13.4	86	7.4	4.7	2.9	97	1.25
95	642608C	44	2	1	3	1	1	2	168	68	24.1	2	2	1	105	11.7	81	8.4	5.2	1.44	101	1.03
96	232814C	56	1	3	3	2	2	2	168	74	26.2	2	2	1	90	15.7	83.6	6.3	4.3	2.4	103	1.19
97	209189F	65	2	1	2	1	2	2	161	48	18.5	1	1	1	165	11	90.6	6.4	4.2	1.4	90	0.92
98	598217D	48	2	1	3	2	2	2	168	68	24.1	2	2	1	105	12.8	89.7	7.1	4.2	1.5	100	0.9
99	350484D	42	1	2	3	1	2	2	165	58	21.3	2	2	1	120	14.5	87.8	7.8	4.8	1.07	87	1.05
100	299882D	41	1	1	3	1	2	2	171	64	21.9	2	1	1	105	14.4	88	7.4	4.5	1.2	102	0.99
101	469889F	37	1	1	3	2	2	1	171	78	25.5	2	2	2		13.9	74	7.3	4.6	2.28	102	0.9
102	881629C	49	2	2	3	2	2	2	156	45	18.5	2	2	1	120	10.7	91.5	7.4	4.6	2.12	79	1.18
103	601373D	49	1	1	3	2	2	1	171	70	23.9	2	2	1	105	12.1	93.5	7.2	4.4	1.3	98	1.27
104	760584D	50	1	5	1	1	2	1	161	50	19.3	2	2	1	135	11.7	87.2	7.8	4.6	3.4	78	0.87
105	650168F	25	1	1	1	1	2	1	165	54	19.8	2	2	1	90	15.6	90.6	8.2	5.1	2.38	89	1.01
106	645631F	24	1	1	3	2	2	2	168	70	24.8	2	2	1	120	15.1	79	7.8	4.7	1.6	99	1.02
107	652734F	41	1	1	1	2	1	1	168	50	18.4	2	2	2		12.1	84	7.7	4.6	3.57	88	1.04
108	649260F	27	1	1	3	1	2	1	175	48	15.7	2	2	1	105	14.4	97.9	7.6	4.8	7.3	85	1.01
109	653434	34	2	6	3	1	2	2	158	53	19	2	2	1	75	11	89	7.	4.5	1.	92	0.96

	F										.8						.4	8		7		
110	652592F	32	1	1	3	2	2	1	170	51	17.6	2	2	2		13.7	91.7	7.5	4.7	2.4	99	1.12
111	654324F	30	1	1	1	1	2	1	165	54	19.8	2	2	1	150	14.5	93.5	7.8	5.1	4.36	86	0.94
112	650279F	36	1	5	3	1	2	2	175	68	22.2	1	1	1	120	14.1	91.5	8.1	5.1	4.2	99	1.1
113	652380F	43	1	2	3	2	2	2	168	43	15.2	2	2	1	135	13.4	81	7.3	4.7	1.4	94	1.37
114	652779F	45	1	1	2	1	1	1	168	70	24.8	2	2	1	105	12.7	89.1	7.2	4.4	7.1	87	1.15
115	759571C	38	2	3	3	1	2	2	156	45	18.5	2	2	1	75	12.3	84.3	7.9	4.7	2.39	84	0.81
116	442578F	26	1	1	1	2	2	1	155	46	18.9	2	2	1	90	15.2	74	8.3	5.2	1.43	94	1.32
117	658435F	51	1	1	3	1	2	1	161	50	19.3	2	2	1		14	78	8	4.9	3.76	102	0.88
118	654819F	34	1	2	1	2	1	1	165	51	19.7	2	2	1	120	11.6	63.9	7.8	5	2.51	93	0.98
119	447661D	36	2	1	3	1	2	2	160	58	22.7	2	2	1	90	12.7	81	8.2	4.6	0.9	93	0.75
120	655203F	47	1	5	3	1	2	2	178	65	20.5	2	2	1	105	14.4	89.2	7.8	4.6	1.2	78	1
121	624256F	27	2	1	3	1	2	1	155	64	27.1	2	2	1	105	12	83.2	7.2	4.4	3.9	94	0.87
122	632882F	29	2	1	2	1	2	2	145	39	18.5	2	2	1	120	11.9	77	8.1	4.4	1.49	86	0.7
123	650852F	48	1	1	3	1	2	1	160	64	25	1	1	1	90	13.2	88	7.4	4.4	1.03	74	0.9
124	621377F	33	1	1	1	1	2	2	168	63	22.3	2	2	2		15.3	95	7.8	5.1	1.42	86	1.04
125	644787F	39	2	1	3	1	1	1	154	48	20.2	2	2	1	165	12.6	86.4	7.6	4.6	2.02	103	0.88
126	662000F	45	1	1	3	1	2	2	165	77	28.3	2	2	1	150	12.4	64.7	7.5	4.5	1.62	115	1.12
127	661998F	55	1	1	3	1	2	1	167	68	24.4	2	2	2		12.6	90.4	8	4.6	0.74	84	0.93

128	338852B	55	2	3	3	1	2	2	154	52	21.9	2	2	1	90	13.4	81	7.3	4.3	2.1	96	0.91
129	641619F	27	1	1	3	1	2	1	160	48	18.7	2	2	1	90	14.8	84.4	8.3	5.2	2.03	94	0.96
130	659441F	29	2	1	3	2	1	1	167	47	16.9	2	2	1	120	12.1	77.1	8.4	5	2.3	91	0.92
131	615582F	45	1	3	2	1	2	2	170	56	19.4	2	2	2		14.7	88.2	7.4	4.8	3.3	110	0.97
132	652684F	44	2	3	2	2	2	2	164	80	29.7	2	2	1	135	9.4	81.4	6.9	4	1.77	106	0.77
133	662068F	29	2	1	3	1	1	1	161	78	30.1	1	1	1	165	11.7	78.3	7.4	3.8	7.7	104	0.9
134	660597F	38	1	6	2	1	2	2	165	65	23.9	2	2	1	120	11.3	60.8	8.3	4.8	1.58	95	1.08
135	661225F	28	2	1	3	1	1	1	158	40	16.1	2	2	2		12.3	85.3	8.5	5	2.81	101	0.89
136	662867F	47	1	5	2	1	1	2	171	58	19.8	2	2	1	165	13.6	95.2	7.8	4.7	3.97	99	0.96
137	297805F	27	2	1	2	2	2	1	165	45	16.5	2	2	1	75	9.8	72.5	7.7	4.6	3.9	83	0.91
138	670476F	35	1	6	1	1	1	2	175	78	25.5	2	2	2		11.9	96.8	7.2	4.8	2.44	89	1.21
139	680064F	21	1	1	3	2	2	1	154	36	15.2	2	2	1	120	15.2	81	7.6	4.6	1.49	77	1.12
140	679319F	47	1	5	1	2	1	2	172	68	13.8	2	2	1	75	13.8	86.6	7.8	4.8	4.54	103	1.3
141	647276F	34	1	5	1	2	2	1	170	92	12.6	2	2	1	105	12.6	84.7	7.4	4.9	2.96	94	1.33
142	673840F	40	1	2	1	1	1	1	168	65	23	2	2	1	150	13.4	89.5	7.8	4.7	1.69	84	1.29
143	672600F	30	1	5	2	2	1	1	170	50	17.3	2	2	1	120	11.9	98	7.6	4.3	3.53	81	1.23
144	684235F	25	1	1	1	1	2	2	168	64	22.7	2	2	2		14.5	93.9	7.6	4.7	2.4	89	0.98
145	667209F	32	1	5	3	1	2	2	170	63	21.8	2	2	1	75	14.1	99	7.7	4.8	3.9	87	1.05
146	672655	34	1	1	1	1	1	1	161	45	17	2	2	1	90	13	83	7.	4.8	1.	84	0.9

	F										.4					.1	.2	8		3		
147	656625F	56	2	5	3	1	2	2	150	55	22.2	2	2	1	150	111.5	92.4	7.3	4.3	1.2	89	0.83
148	677955F	27	1	1	1	1	2	1	170	45	15.6	1	1	1	90	133.3	73.1	7.7	4.4	3.6	101	1.01
149	502834D	50	2	1	3	1	1	2	165	60	22	2	2	1	90	122.2	91.5	7.5	4.4	2.5	90	0.62
150	678726F	29	2	1	2	1	2	1	165	50	18.9	2	2	1	105	131.1	86.6	8.1	4.6	2.08	102	0.89
151	683050F	35	1	1	1	1	2	1	174	95	31.4	1	1	1	105	131.1	87.6	7.3	4.2	1.23	99	1.06
152	673316C	39	2	1	3	2	2	1	160	47	18.4	2	2	1	105	111.4	88.8	7.4	4.6	3.47	83	0.89
153	680720F	37	1	1	3	1	1	1	165	56	20.6	2	2	1	120	144.7	90.9	8.1	5	0.78	110	0.95
154	679703F	26	1	1	3	1	2	1	161	49	18.9	2	2	1	60	155.3	96	7.3	4.9	1.73	85	1.1
155	679178F	44	1	2	3	1	2	1	161	78	30.1	2	2	1	75	144.9	90.7	7.7	4.5	6.2	99	1.12
156	685391F	45	2	1	3	2	2	1	156	65	26.7	2	2	1	135	122.2	91	7.5	4.7	1.15	92	1
157	662836F	38	2	1	1	2	2	1	162	72	27.4	2	2	1	105	111.3	85.1	7.6	4.6	5.01	86	0.9
158	679859F	34	1	1	2	1	2	2	162	70	26.7	2	2	2		132.2	89.7	7.3	4.5	4.59	79	1.06
159	622740F	41	1	3	1	2	2	2	170	74	25.6	2	2	1	105	133.9	93.3	7.4	4.4	2.1	96	1.27
160	663499F	48	1	1	2	1	1	2	170	60	20.8	2	2	1	135	122.6	77.8	8.1	4.7	3.31	101	1.12
161	648728F	29	1	1	1	2	1	1	163	49	18.4	2	2	1	120	144.4	92.6	7.9	5.2	1.65	94	0.92
162	673987F	40	2	1	3	2	2	2	155	56	23.3	2	2	2		10	84.3	7.4	4	2.48	101	1.02
163	677763F	38	1	1	1	2	2	1	175	71	23.2	2	2	2		142.2	96.8	6.9	4.6	1.42	97	0.92
164	778153D	25	2	3	3	1	1	2	154	48	20.2	2	2	1	165	136.6	88.1	7	4.3	1.27	86	0.55